Vol. 50, No. 10, April, 1961

Russian Original Dated October, 1960

BULLETIN OF EXPERIMENTAL BIOLOGY AND MEDICINE

БЮЛЛЕТЕНЬ ЭКСПЕРИМЕНТАЛЬНОЙ БИОЛОГИИ И МЕДИЦИНЫ

BYULLETEN' ÉKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

TRANSLATED FROM RUSSIAN

CONSULTANTS BUREAU

The first volume in

The | International Behavioral Sciences | Series

under the editorship of Joseph Wortis, M.D.

SLEEP THERAPY IN THE NEUROSES

by B. V. Andreev, M.D.
Pavlov Institute of Physiology,
Academy of Sciences of the USSR.

TRANSLATED FROM RUSSIAN
by Basil Haigh,
M.A., M.B., B. Chir.

This continuing series of translated monographs will include important works which are not normally available to the Western psychiatrist, psychologist, or social scientist. It will make it possible for the English-speaking professional to keep abreast of the latest trends and advances in the behavioral sciences in the Soviet Union, Asia, Latin America, and Europe.

In SLEEP THERAPY IN THE NEUROSES, the author presents the results of clinical and experimental research performed since 1946 at the Pavlov Clinic for Nervous Diseases. (Pavlov's concept of protective inhibition was based on his conviction that sleep often permits restitution of function when the Central Nervous System has been exhausted by stress.) In an attempt to produce sleep without toxic manifestations, the Soviet researchers used hypnosis, suggestion, and certain physical hypnogenic stimuli, which helped keep the dosage of sedative drugs to a minimum. Actography was employed to measure the duration of therapeutic sleep, making it possible to record the transition from waking to sleep and vice versa, and to evaluate the efficacy of various measures in prolonging sleep. In a proportion of patients the higher nervous activity was investigated by experimental methods, which served as a valuable supplement to the clinical observations.

CONTENTS

Introduction
Modern Views on Neuroses
  Protective Inhibition
A Brief Outline of the History of the Method of Sleep Therapy
The Indications for Sleep Therapy in Neuroses
The Investigation of Sleep by the Method of Actography
Sleep Therapy in the Neuroses
  Composition of the Patients
  Method of Treatment
  Preparation for Treatment
  Forms of Therapeutic Sleep
  Program for the Day
  The Environment and Its Importance
  Physical Hypnogenic Factors
  The Use of Hypnotic Drugs
  The Use of Inert Substances (Hypnotic Substitutes) as Conditioned Reflex Stimuli
  The Use of Hypnosis and Suggestion
  The Daily Duration of Sleep and Its Characteristics
  The Length of the Course of Treatment
  The Changeover from Sleep Therapy to the Ordinary Routine
  Subsequent Period of Treatment
  The Results of Treatment
  Follow-up
Conclusion
Supplement: Technical Details for the Proper Organization of Sleep Therapy
Literature Cited

114 pages $8.50

CONSULTANTS BUREAU
227 W. 17 ST., NEW YORK 11, N. Y.

EDITORIAL BOARD OF BYULLETEN' EKSPERIMENTAL'NOI BIOLOGII I MEDITSINY

V. V. Parin *(Editor)*
A. Ya. Alymov
V. N. Chernigovskii
S. Ya. Kaplanskii
G. N. Kryzhanovskii *(Secretary)*
L. D. Liozner
I. N. Maiskii
V. A. Negovskii
V. A. Sanotskii
L. L. Shik
A. A. Solov'ev
N. N. Zhukov-Verezhnikov *(Acting Editor)*

BULLETIN OF

EXPERIMENTAL BIOLOGY AND MEDICINE

A translation of BYULLETEN' EKSPERIMENTAL'NOI BIOLOGII I MEDITSINY, a publication of the Academy of Medical Sciences of the USSR

(Russian Original Dated October, 1960)

Vol. 50, No. 10, pp. 985-1096 April, 1961

CONTENTS

	PAGE	RUSS. PAGE
PHYSIOLOGY		
The Influence of Efferent Nerves to the Thyroid Gland on Thyroid Function. M. G. Amiragova	985	3
The Relation Between Reflex Changes of the Systemic and Local Circulation. V. V. Frol'kis and I. V. Shchegoleva	989	7
Periodic Motor Activity of the Stomach During Digestion. V. G. Startsev	993	12
Change of the Functional State of the Peripheral Neuromuscular Apparatus During the Formation of a Dominant in the Spinal Cord. Communication I. Influence of Spinal Cord Dominant on the Parameters of Excitability of the Nerve and Muscle. F. D. Sheikhon and G. V. Nikishin	997	16
The Spatial Distribution of Electrical Activity in the Cerebral Cortex of Man and Animals. K. K. Monakhov	1004	23
PATHOLOGICAL PHYSIOLOGY AND GENERAL PATHOLOGY		
The Effect of Hypoxic Hypoxia and Low Barometric Pressure on the Human Electrocardiogram (Vector Analysis). V. N. Alifanov.	1009	29
Respiratory Electromyograms During Resistance to Breathing Before and After Vagal Section. Yin Chi-chang	1013	34
The Effect of Artificial Pneumothorax on Afferent and Efferent Impulses in the Respiratory Pathways. M. I. Vinogradova	1019	40
Blood Coagulation in C-Avitaminosis. Communication III. The Effect of Vitamins C, P, B_{12}, K, and Folic Acid on the Thromboplastic Activity of the Blood of C-Avitaminotic Guinea Pigs. G. V. Andreenko and N. P. Sytina	1024	46
Hematopoiesis and Vitamin B_{12} Content in Dogs After Gastrectomy and in Experimental Hepatitis. O. A. Korolenko	1026	48
Changes in the Blood System Associated with Denervation of the Duodenum. R. A. Durinyan	1030	53
The Involution of Experimental Cholesterol Atherosclerosis in Dogs. A. N. Fedoseev	1034	58
On the Question of Changes in the Acid-Base Balance and the Redox Processes in Peritonitis Associated with Blood Loss. E. M. Neiko and L. Ya. Fishchenko.	1037	62
BIOCHEMISTRY AND BIOPHYSICS		
The Characteristics of the Nuclear Nucleoproteins of Some Experimental Tumors and of Chick Embryos. I. B. Zbarskii and L. P. Ermolaeva	1039	64
The Problem of the Effect of Parasympathetic Denervation on the Protein Metabolism of the Submaxillary Salivary Glands of the Cat. G. V. Chernysheva	1042	67
The Effect of Carbon Dioxide Gas on the Content of Ammonia, Glutamine, and Urea in the Blood of Animals After Injection of Solutions of Ammonium Chloride. N. B. Kozlov	1048	73

Annual Subscription $20.00
Single Issue 10.00

© 1961 Consultants Bureau Enterprises, Inc., 227 West 17th St., New York 11, N. Y.
Note: The sale of photostatic copies of any portion of this copyright translation is expressly prohibited by the copyright owners.

CONTENTS (continued)

	PAGE	RUSS. PAGE
The Effect of High Altitude on the Protein Composition of Human Blood. E. P. Smolichev	1053	78
Mitogenetic Radiation of the Neuromuscular System as a Method of Analyzing Its Molecular Substrate. Communication II. On the Application of the Concept of Molecular Substrate Regulation in the Resting Muscle. A. A. Gurvich	1056	82
PHARMACOLOGY		
Age Sensitivity to Barbiturates and Its Dependence on the Development of the Hypophyseal-Adrenal System. I. V. Markova	1060	87
MICROBIOLOGY AND IMMUNOLOGY		
Sensitizing Properties of the Parenchymatous Organs of Kittens and White Mice Infected with Dysentery Bacilli and Streptococci. M. V. Zemskov	1064	92
On the Immunologic Characteristics of Serum Proteins. Part 1. Induction of Anaphylaxis by Isolated Fractions of Blood Serum Proteins. Yu. S. Tatarinov	1069	97
ONCOLOGY		
The Effect of Antironidase Serum of Goats on the Metastasization of Brown-Pearce Carcinoma in Rabbits. I. N. Maiskii and N. A. Kozlova	1072	101
A 5-Hydroxytryptamine from Hippophae rhamnoides as an Antitumor Preparation in Experiments on Animals. E. Ch. Pukhal'skaya, M. F. Petrova, and G. P. Men'shikov	1076	105
EXPERIMENTAL BIOLOGY		
The Migration of Sr^{90} Along the Biological Chain Plant-to-Sheep-to-Fetus. L. A. Buldakov and Yu. I. Moskalev	1081	111
Regeneration of the Parotid Gland in the Rat. A. G. Babaeva	1084	113
24-Hour Rhythm of Cell Multiplication in Rat Epidermis During Healing of Skin Wounds. M. T. Gololobova	1088	118
Development and Regression of Hexestrol-Induced Changes in the Spermatic Tract of the Rabbit. A. G. Bobkov	1091	122

THE INFLUENCE OF EFFERENT NERVES TO THE THYROID GLAND ON THYROID FUNCTION

M. G. Amiragova

Laboratory of General Physiology (Head-Active Member AMN SSSR V. N. Chernigovskii)
Institute of Normal and Pathological Physiology (Dir.-Active Member AMN SSSR V. N. Chernigovskii)
AMN SSSR, Moscow
(Presented by Active Member AMN SSSR V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 3-7, October, 1960
Original article submitted December 31, 1959

Published data concerning the importance of the innervation of the thyroid gland for its function are contradictory. Recently, however, more and more investigators have come to the conclusion that the efferent nerves to the thyroid gland are vasomotor, and have no direct secretory function [3, 4, 5].

In view of these data, we were interested in finding out how the thyroid gland, when it is deprived of its nervous connections, functions under conditions of so-called emotional excitation.

Our premise was that if the nervous component in the transmission of cortical impulses to the periphery is of substantial physiological significance, the secretory activity of the denervated gland under these conditions ought to differ radically from the normal.

EXPERIMENTAL METHODS

Eight dogs with denervated thyroids were used in these experiments. The controls were three unoperated animals. The thyroid glands were denervated in the standard way. All the nerve branches from the superior laryngeal nerve, the superior cervical ganglion, and the recurrent nerve to the thyroid gland were excised over a length of 1-2 cm. The blood vessels supplying the thyroid were carefully painted (until they turned white) with 5% phenol. Experiments were performed on these animals 1-4 weeks after the operation. The level of radioiodine (I^{131}) in the thyroid was taken as the indicator of its functional state. We shall not describe in detail the method used for determing radioiodine in the thyroid gland in the whole animal while the latter is working in the conditioned reflex chamber, since we have set forth this method in earlier publications [1, 2].

The functional state of the central nervous system was altered by the presentation of conditioned defense stimuli. Conditioned reflexes were developed to the sound of a metronome at 120 beats per minute. The unconditioned stimulus was an electrical current from an inductorium. The conditioned stimulus was given at five-minute intervals, ten times in each experimental session. The conditioned stimulus was applied alone for 20 sec each time. I^{131}, 0.7 μC/kg, was given by mouth, with milk. The experiments were performed 72 hr after the administration of I^{131}. Thyroid gland radioactivity was determined after each conditioned stimulus and for four hours after the last conditioned stimulus was applied.

EXPERIMENTAL RESULTS

The reactions of normal and denervated thyroid glands to conditioned defense stimuli were studied. The experiments showed that these stimuli elicit the release of hormones from both innervated and denervated thyroids. As may be seen from the data shown in Table 1, in all the experiments we found a sizable drop in the I^{131} level in the denervated thyroid gland. These experiments give evidence that thyroid glands deprived of their nervous connections lose none of their capacity to react to impulses originating in the central nervous system. It can not be said, however, that the reactions of denervated glands are in no way different from those of normal glands. We noted several characteristic features in the reactions of glands deprived of their innervation.

First, the reactions of denervated glands were of greater amplitude than those of intact glands. As is apparent from the figure, denervated thyroids reacted to defense-conditioned stimuli with a marked reduction in the level of radioactivity. At the same time, these reactions were distinguished by their low mobility, in that they displayed very slight variations in the level of radioactivity during the course of an experiment.

Secondly, the reactions of denervated thyroids lasted longer than those of normal glands. It was observed that the level of radioactivity of the denervated gland, after a 44.4% reduction at the outset of an experiment, thereupon remains constant throughout the entire experiment (4-5 hr), showing only slight variations in one direction or another. The reactions of normal thyroids

TABLE 1. Effect of Conditioned Defense Activity on Radioiodine Content in Dog Thyroid After Denervation*

Date of experiment, 1959	Radioactivity in intervals following individual conditioned stimuli — number of preceding conditioned stimulus										Radioactivity following conditioned stimulation — time (minutes)							
	1	2	3	4	5	6	7	8	9	10	15	45	75	105	135	165	195	225
Aug. 21	—24.1	—23.5	—29.9	—28.2	—26.9	—19.3	—23.4	—24.5	—24.9	—26.6	—27.3	—26.3	—27.1	—22.7	—20.3	—21.9	—22.4	—22.4
" 25	—39.3	—21.5	—36.4	—36.6	—32.9	—34.5	—35.4	—35.0	—36.7	—	—36.0	—35.5	—37.9	—36.2	—39.7	—36.7	—38.2	—34.5
" 27	—42.5	—41.9	—41.9	—40.9	—37.1	—40.5	—35.8	—38.1	—42.9	—37.7	—38.5	—30.6	—35.0	—33.8	—31.8	—35.8	—33.4	—33.8
" 22	—26.8	—22.5	—21.8	—15.6	—23.1	—21.8	—22.0	—21.4	—22.5	—24.2	—18.2	—21.2	—19.9	—20.7	—21.6	—20.3	—22.0	—21.4
" 24	—23.3	—26.9	—23.3	—30.5	—20.4	—33.2	—34.8	—39.5	—30.5	—34.8	—19.1	—33.2	—17.5	—36.8	—25.3	—27.3	—29.9	—29.9
" 26	—41.0	—45.7	—37.8	—35.1	—39.8	—46.1	—45.7	—39.0	—42.3	—44.5	—39.0	—43.0	—41.4	—42.3	—40.6	—44.1	—39.0	—35.1
Sept. 8	—32.9	—46.5	—38.9	—37.9	—39.2	—35.8	—39.4	—37.5	—33.4	—31.9	—41.7	—45.3	—40.6	—37.9	—41.1	—42.4	—37.0	—
" 10	—33.2	—32.4	—33.6	—30.8	—17.3	—18.3	—19.5	—26.4	—22.0	—	—24.3	—15.1	—12.7	—19.7	—15.9	— 8.2	—	—
" 12	—44.4	—43.5	—44.4	—45.3	—45.9	—42.3	—45.9	—43.7	—42.3	—47.2	—43.1	—41.2	—43.5	—44.4	—46.5	—46.0	—44.0	—44.4

*Results of experiments are expressed as percent of initial radioactivity.

TABLE 2. Effect of Conditioned Defense Activity on Radioiodine Content in Normal Dog Thyroid*

Date of experiment, 1959	Radioactivity in intervals following individual conditioned stimuli — number of preceding conditioned stimulus										Radioactivity following conditioned stimulation — time (minutes)						
	1	2	3	4	5	6	7	8	9	10	15	45	75	105	135	165	195
Sept. 9	—12.0	—18.0	— 9,8	—13,5	—10,7	— 7,2	— 2,3	— 4,8	— 8,5	— 3,9	— 3,9	— 4,8	— 5,7	— 3,9	— 3,2	—	—
" 11	—26,7	—21,8	—20,0	— 9,6	—14,7	—15,4	— 8,3	— 6,9	— 8,7	— 5,5	— 8,0	—10.5	— 9,1	—11.5	—12,6	— 9,8	—
" 16	—27,8	—26,6	—33,0	—27,8	—15.0	—22,5	—14,7	—20,5	—11,3	—21,2	— 7,4	— 8,9	— 9,8	—12,5	—16,8	—13,5	— 8,3
" 20	— 9,0	—11,7	— 9,5	— 7,4	—10,6	— 8,4	— 6,8	— 5,7	— 4,1	— 3,6	— 6,3	+ 2,9	— 5,7	— 5,7	— 4,1	— 9,0	— 7,9
" 22	— 9,0	—16,0	—17,1	—13,1	—11,9	—15,4	— 9,0	—10,2	—20,0	— 5,0	—28,7	—10,2	+ 0,2	— 2,7	— 2,7	— 3,2	— 3,8
" 24	—32,2	—22,3	—28,5	—28,3	—24,1	—28,5	—18,5	—24,1	—27,3	—12,9	—11,7	— 3,6	—11,0	— 1,7	— 5,8	— 5,8	—
Oct. 16	—20,9	—18,9	—25,4	—22,7	—30,1	—27,9	—30,0	—23,6	—28,4	—27,7	—32,7	—26,9	—30,4	—23,8	—32,2	—30,4	—29,3
" 19	— 8,8	— 5,8	— 2,8	— 5,8	— 8,3	— 8,3	— 5,3	— 9,8	— 9,8	—11,3	—16,9	—11,8	—11,3	—10,8	—14,4	—13,9	—14,9
" 19	— 3,6	+ 0,3	— 8,4	— 8,8	— 8,8	—15,7	—15,4	—18,8	—19,8	—	—15,7	—11,2	— 9,8	—12,6	+ 9,5	+ 7,0	+ 9,5
" 21	—17,8	—17,4	—20,0	—17,8	—20,4	—22,6	—20,7	—20,0	—22,6	—11,9	—23,8	—17,4	—14,1	—20,7	—17,1	—13,4	—11,1

*Results of experiments are expressed as percent of initial radioactivity.

were different; as a rule, their radioactivity level approached the normal physiological range by 1-2 hr after the end of conditioned stimulation, and only occasionally remained low for longer periods. Thus the experiments showed that the reactions of denervated glands differ from normal in having greater amplitudes and less lability.

With regard to the mechanism of this phenomenon, it should be observed that with the setup used in these experiments the question is complicated by the presence of the vasomotor innervation of the thyroid. Since there unquestionably exists an intimate relation between the gland's blood supply and its secretory activity, the change in the diameter of its vessels and the counsequent filling withblood after denervation of the gland must naturally affect the secretion process. And if we try to understand what it is in the reaction of the denervated gland that depends on the damage to the vasomotor fibers, it seems to us that, first and foremost, the inertness and extreme duration of the reaction must be attributed to this cause.

It is known that vasomotor reactions are especially pronounced in emotional excitation, and can not be without effect on organ activity. And in this case we may assume that the monotonous and drawn-out character of reactions observed after denervation is to be understood from this standpoint.

In the experiments on intact animals we often observed large variations in the level of radioactivity of the gland; we saw that during the course of an experiment the radioactivity of the gland first fell, then returned to the original level. This is well shown in the experiments of September 9, 11, 16, and 22, all of which are presented in Table 2. It is not impossible that these variations in thyroid activity during the defense reaction reflect the participation of vasomotor fibers. It is no coincidence that after denervation of the gland these variations almost completely disappeared.

The reactions of denervated glands, as we have indicated above, were more marked than those of intact glands. We felt that the cause of this phenomenon lies in the sensitization of denervated thyroid cells to epinephrine.

If this hypothesis is correct, then a reduction of the blood epinephrine concentration should cause a weakening of the reaction of denervated thyroids.

In order to limit the entrance of epinephrine into the blood, after denervating the thyroids we performed a second operation in which we completely removed the adrenal gland on one side and excised portions 1-2 cm long of the large and small splanchnic nerves, to eliminate reflex secretion of epinephrine.

As may be seen from the figure, under the influence of the conditioned defense stimuli the thyroid glands of these animals secrete less actively into the blood than do the glands of animals after the first operation. The figure shows that the maximum change in the radioactivity of the thyroid gland after the first operation is -47.2%, and in dogs after both operations it is only -18%. This fact confirms our hypothesis that the reaction of denervated thyroids seen in these experiments is the result of sensitization of the gland cells to epinephrine.

Our experiments showed that the efferent nerves to the thyroid gland ensure the completest possible adaptation of the gland to altered environmental conditions.

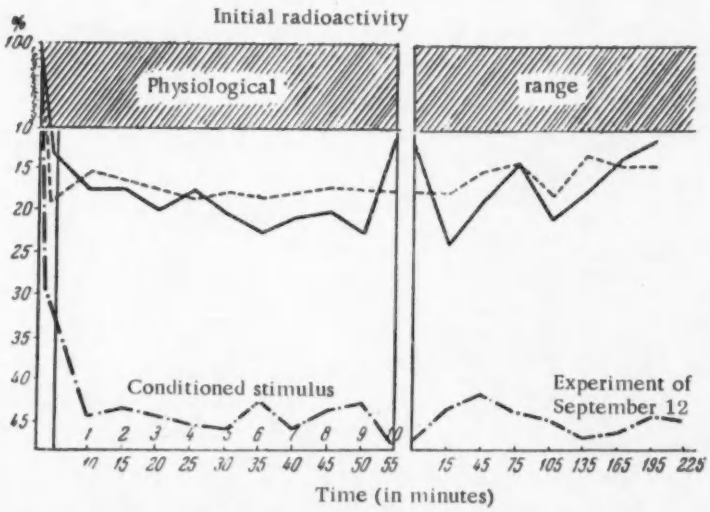


Figure. The effect of conditioned defense activity on radioiodine content in dog thyroid. —— normal; ---- after denervation on the thyroid gland and one adrenal, and extirpation of the other adrenal; —·—· after denervation of the thyroid gland.

SUMMARY

The author studied the reaction of the denervated thyroid gland to conditioned defense stimuli. The functional state of the gland was determined from changes in its content of radioactive iodine (I^{131}). Experiments on dogs with denervated thyroids showed that after denervation cortical influences still reach the thyroid gland. But the reaction of the denervated thyroid is different from that of the normal thyroid: it is more pronounced and more prolonged than normal.

Dogs subjected to a second operation-removal of one adrenal gland and denervation of the other-reacted in a manner suggesting that the response of denervated thyroids is due to sensitization of the gland cells to epinephrine, as well as to denervation of the blood vessels of the thyroid.

LITERATURE CITED

1. M. G. Amiragova, Abstracts of the All-Union Scientific and Technical Conference on the Use of Radioactive and Stable Isotopes and Radiation in the National Economy and Science [in Russian] (Moscow, 1957) p. 164.
2. M. G. Amiragova, Fiziol. Zhur. 1, 65 (1957).
3. K. Brown-Grant, G. W. Harris, and S. Reichlin, J. Physiol. 136, 364 (1957).
4. E. Gellhorn, Physiological Foundation of Neurology and Psychiatry (London, 1953).
5. U. Söderberg, Short Term Reactions in the Thyroid Gland (Stockholm, 1958).

THE RELATION BETWEEN REFLEX CHANGES OF THE SYSTEMIC AND LOCAL CIRCULATION

V. V. Frol'kis and I. V. Shchegoleva

Department of Normal Physiology (Head-Acad. G. V. Fol'bort*)
of the Kiev Medical Institute (Dir.-Docent I. P. Alekseenko)
(Presented by Active Member AMN SSSR B. N. Man'kovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 7-12, October, 1960.
Original article submitted November 16, 1959

The problem of the relationships between local and systemic changes in circulation can properly be considered one of the central problems in the physiology and pathology of the cardiovascular system.

Present-day physiology has collected an enormous amount of information concerning the characteristics of the reflex mechanisms that ensure adjustment of the cardiovascular system to the constantly changing conditions of the organism's activity. But it often has not been taken into account that the systemic hemodynamic indices are the net result of extremely diverse local circulatory changes. This state of affairs has been discussed repeatedly by clinicians.

It is therefore not difficult to understand the interest aroused by the problem of the relationship between the systemic and the regional circulation.

The existence of various interrelations between the systemic and local changes in circulation has already been considered in the work of V. N. Chernigovskii [15], M. E. Marshak [3, 4], V. M Khayutin [11, 12, 13], T. S. Lagutina[1,2], V.L. Tsaturov[14,] and N.K. Saradzhev[5].

V. N. Chernigovskii and V. M. Khayutin developed the conception that individual vascular regions are represented in the vasomotor center. It is their view that the preferential excitation or inhibition of individual groups of neurons connected with one vascular bed or another may arise as a result of activity of part of the vasomotor center.

In elucidating the mechanisms of the systemic and local circulation it is essential, in our opinion, to make a simultaneous comparison of the reactions of several regional vasculatures with the changes in systemic blood pressure, and under a variety of conditions of stimulation of various receptor zones. There is no need for special proof that relationships between the systemic and local circulation can be established by studying the vascular reactions of organs with different functional significance.

METHODS

The experiments were performed on cats and rabbits under urethan (cats) and chloral hydrate (rabbits) anesthesia. Changes in systemic blood pressure and respiration were recorded simultaneously, as well as the activity of the ear vessels and those of the kidney and hindlimb. The systemic blood pressure was recorded from the carotid artery with mercury and spring-type manometers, and respiration with a tracheotomy tube connected to a Marey tambour. The activity of the vessels of the ear, kidney, and hindlimb was studied by the method of perfusion with Ringer-Locke solution to which N. G. Belen'kii's therapeutic serum was added in a 4 : 1 dilution to reduce edema formation.

The perfusion fluid entered the vessels of the organ under constant pressure (80 cm of water) and at constant temperature through a cannula inserted into the corresponding artery (the large auricular, renal, and femoral arteries, respectively), and left through a cannula from the corresponding vein.

The reaction of the vessels was given by the number of drops leaving by way of the vein; as they fell, the drops closed the contacts of a circuit containing a storage battery, a polarizing relay, and an electrical marker.

We studied the changes in the systemic and local circulation accompanying stimulation of receptors of the carotid sinus and the upper respiratory tracts and the tibial and aortic nerves, and also those accompanying transient asphyxia.

RESULTS

The data obtained testify to the extreme diversity of the relationships in the changes of local and systemic vascular tone under reflex influences on the cardiovascular system. To put it differently, one type of change in the systemic blood pressure may result at different times from entirely different distributions of local vascular reactions. But one general rule emerges in the whole diversity of regional changes: there is coordination between the reactions of different vascular beds, directed toward the restoration of the original level of systemic blood pressure.

---

* Deceased

Application of stimuli of different intensities and durations to receptor fields and nerves allows us to demonstrate certain stages in the relationships between local and systemic hemodynamic changes.

In a number of experiments, stimulation of the tibial or depressor nerves with a current of subthreshold intensity did not result in changes in the systemic blood pressure, but the tone of individual vascular beds did change.

Even more diverse relationships were found between local reactions accompanying marked changes in the systemic blood pressure. We sometimes observed simultaneous constriction and dilatation of the vessels of different regions, while in other experiments the changes were just the opposite. So, for example, on stimulation of the depressor nerve the reduction in systemic arterial pressure was sometimes accompanied by constriction of the ear vessels and dilatation of the vessels of the hindlimb. On the other hand, some vascular beds (the renal vessels) did not react to stimulation of the depressor nerve.

The reactions of local vascular beds did not always remain constant, even in response to stimulation of the same receptor field. Thus, following stimulation of the tibial nerve we were able to observe constriction of the renal vessels and dilatation of the vessels of the ear or hindlimb; but in other experiments we found the opposite effect. In a large fraction of the experiments, particularly following the application of intense stimuli, we observed a uniform type of change in vascular tone. These experiments confirm the conclusion, drawn by V. M. Khayutin, that there are different connections between different receptor fields and vascular beds. Comparison of the reactions of the vessels of different beds shows that following stimulation of the upper respiratory tracts (inhaling ammonia vapors) and the tibial nerve (induction current), the vessels of the ear and hindlimb are particularly reactive (Fig. 1). In response to stimulation of the carotid sinus receptors (clamping the carotid artery) the greatest changes occur in the renal vessels, and the tone of the ear vessels and those of the hindlimb remains almost unchanged.

The reactions of individual vascular beds display specific differences. Thus, the reaction of the vessels of the hindlimb to stimulation of various receptor fields is marked by a distinct diphasic character. It is well known that stimulation of the tibial nerve is followed by a pressor reaction. But at the beginning of stimulation the hindlimb vessels dilate, as shown by a brief increase in the outflow rate, and then a pronounced vasoconstriction ensues.

This sort of diphasic behavior was not observed in the ear vessels, but here the latent period of the reac-

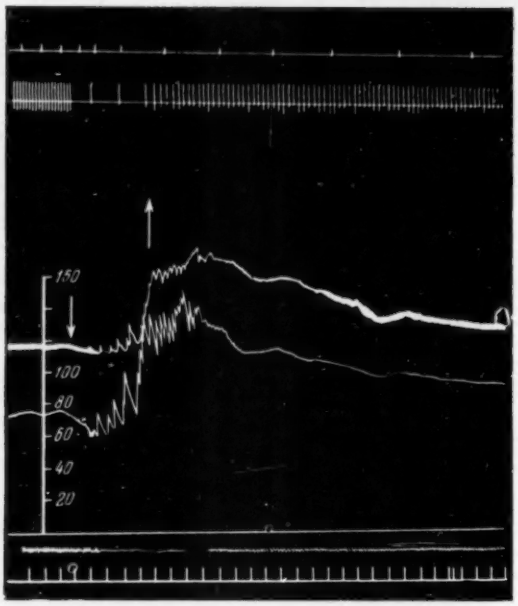


Fig. 1. Changes in systemic blood pressure and tone of vessels of hindlimb and ear in rabbit upon stimulation of upper respiratory tracts with ammonia fumes. Meaning of curves (from top): outflow from vessels of hindlimb and ear, in drops; recording of arterial pressure with membrane manometer and with mercury manometer; zero line for mercury manometer; pneumogram; time marker (5 sec). Arrows point to beginning and end of stimulation.

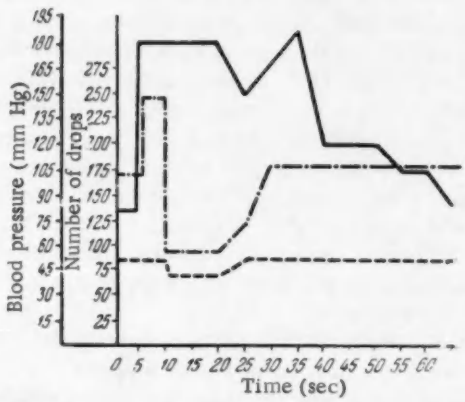


Fig. 2. Changes in systemic arterial pressure and tone of vessels of hindlimb and ear in the rabbit, following stimulation of the tibial nerve. Explanation: abscissa is time (in seconds); ordinate is magnitude of systemic blood pressure (in mm Hg) and number of drops; solid line-changes in systemic arterial pressure; dots and dashes-number of drops flowing out of hindlimb; dotted line-number of drops flowing out of ear vessels.

tion was longer; thus, during the dilatation of the hindlimb vessels the ear vessels did not react (Fig. 2). In mild reactions of the systemic arterial pressure, one can see the variations in the tone of vessels in individual organs.

It is common knowledge that when the application of a stimulus is prolonged the reflexes to the cardiovascular system become weakened-this is known as adaptation of reflex reactions (V. N. Chernigovskii and V. M. Khayutin [16], V. M. Khayutin [10], and V. V. Frol'kis [6, 8, 9]). Study of the changes in local vascular reactions in the course of adaptation enables us to approach one of the components of the mechanism of adaptation.

Adaptation of reflexes proceeds differently for different vascular beds.

Moreover, the vascular reactions of individual organs frequently return to normal before the arterial blood pressure returns to its original level. Restoration of the original level of arterial blood pressure during adaptation of reflexes may occur as a result of compensatory reactions of individual vascular beds. In Fig. 3 an experiment is presented in which "adaptation" of a reflex was observed during prolonged stimulation of the tibial nerve. In response to stimulation, the vessels of the ear and hindlimb constricted, but while the background of systemic hypertension continued we observed a marked dilatation of the ear vessels: the number of drops leaving the ear per unit time increased sharply. This dilatation occurred at a time when the vessels of the hindlimb were still constricted.

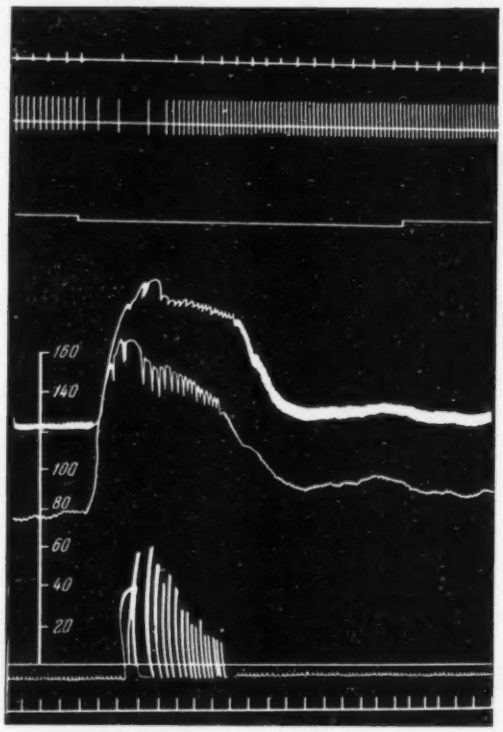


Fig. 3. Changes in systemic arterial pressure and tone of hindlimb and ear vessels in the rabbit during prolonged stimulation of tibial nerve. Meaning of curves (from top): number of drops leaving hindlimb vessels; number leaving ear vessels; stimulus marker; systemic arterial pressure recorded with membrane manometer; same, recorded with mercury manometer; zero line for mercury manometer; pneumogram; time marker (5 sec).

Comparison of the reactions of the systemic and local circulation discloses the extreme diversity and complexity of the changes in the tone of different vascular beds during displacements of the same type in the magnitude of the systemic blood pressure. While the level of arterial pressure remains constant we may observe variations in vessel diameters in only one vascular bed, or opposing shifts-constriction and dilatation-in the reactions of several vascular beds; and we may see numerous variations on the change in regional blood flow while systemic blood pressure rises or falls. The complex relationships of the reactions of individual vascular beds are an important compensatory mechanism directed toward the maintenance of a constant level of hemodynamics.

The reflex changes described above in the blood flow in individual vascular beds confirm the thesis of V. N. Chernigovskii [15] and V. M. Khayutin [12] concerning fractional activity of the vasomotor center. The power of the central mechanisms that regulate the circulation becomes even more evident when we consider the connection of the vascular reactions with changes in the activity of the heart.

At the present time there is reason to consider the whole complex system of central regulation of the circulation as a unit. From this standpoint, a useful view is the view developed by one of us [7] of the hemodynamic center as the complex aggregate of neurons involved in the central regulation of the heart and vessels.

Its activity ensures the diverse changes in the regional circulation, as well as the complete regulation of the cardiovascular system. The concept of the complex dynamics of nervous processes in the hemodynamic center explains the unity of systemic and local changes in blood flow, and shows their connection with changes in cardiac activity.

We can interpret the adaptation of cardiovascular reflexes from the same standpoint. The observed differences in recovery of the tone of various vascular beds, and the development of reactions of opposite character, both confirm the conclusion that inhibition of various degrees and depths develops in the individual parts of the hemodynamic center during adaptation of reflexes. The adaptation of cardiovascular reflexes

may be brought about in some cases predominantly through widespread irradiation of inhibition in the hemodynamic center, and in others, through deepening of inhibition in various groups of central neurons.

SUMMARY

The authors studied the changes in the systemic blood pressure and the tone of blood vessels of the ear, kidney, and hindlimb in rabbits and cats. It was shown that a given type of change in the systemic blood pressure may be caused by different interrelationships between the vascular reactions of the organs. During adaptation of cardiovascular reflexes, the tone of different vascular beds varied differently.

LITERATURE CITED

1. T. S. Lagutina, Byull. Éksp. Biol. Med., 7, 3 (1957).†
2. T. S. Lagutina, Byull. Éksp. Biol. Med., 1, 3 (1959).†
3. M. E. Marshak, Abstracts of Conference on the Circulation [in Russian] (Kiev, 1959).
4. M. E. Marshak, Vestnik Akad. Med. Nauk SSSR, 9, 36 (1959).
5. N. K. Saradzhev, Byull. Éksp. Biol. Med., 2, 49 (1958).†
6. V. V. Frol'kis, in: Higher Nervous Acticity and Corticovisceral Interrelations [in Russian](AN USSR, 1955).
7. V. V. Frol'kis, Reflex Regulation of the Activity of the Cardiovascular System [in Russian] (Kiev, 1959).
8. V. V. Frol'kis and A. V. Frol'kis, Fiziol. Zhur. 42, No. 10 (1956).
9. V. V. Frol'kis and A. V. Frol'kis, in: Processes of Fatigue and Recovery of the Organism's Activity [in Russian] (Kiev, 1958).
10. V. M. Khayutin, Materials on the Mechanism of Adaptation of Unconditioned Interoceptive Reflexes [in Russian] (Leningrad, 1952).
11. V. M. Khayutin, Byull. Éksp. Biol. Med., 10, 18 (1958).†
12. V. M. Khayutin, Transactions of the Ninth All-Union Congress of Physiologists [in Russian] (Minsk, 1959).
13. V. M. Khayutin and V. L. Tsaturov, Byull. Éksp. Biol. Med., 2, 17 (1959). †
14. V. L. Tsaturov, Byull. Éksp. Biol. Med., 9, 3 (1959). †
15. V. N. Chernigovskii, Abstracts of Conference on the Circulation [in Russian] (Kiev, 1959).
16. V. N. Chernigovskii and V. M. Khayutin, in: Nervous Regulation of the Circulation and Respiration [in Russian] (Moscow, 1952) pp. 8-22.

---

†Original Russian pagination. See C. B. translation.

PERIODIC MOTOR ACTIVITY OF THE STOMACH DURING DIGESTION

V. G. Startsev

Department of Normal Physiology (Head-Prof. E. F. Larin)
of the Tomsk Medical Institute (Dir. - Prof. S. P. Khodkevich)
(Presented by Active Member AMN SSSR V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 10, pp. 12-16, October, 1960
Original article submitted May 18, 1959

Periodic "hunger" contractions were first studied in detail by V. N. Boldyrev [1], along with the periodic secretion and motor activity of other parts of the alimentary tract. According to Boldyrev, periodic activity of the stomach is absent during digestion. Other conditions that stop periodic function of the stomach are sham and natural feeding, and acid gastric secretion.

If food enters the stomach without passing through the oral cavity, i.e., through a gastric fistula, the "hunger" contractions do not disappear. Moreover, milk, fluid egg albumin, or neutral fat poured into the stomach passes into the intestine in large amounts. On the other hand, these fluids are retained in the stomach for half an hour or so if they are introduced into it in the absence of "hunger" contractions [11]. Although the absence of periodic gastric activity during digestion has since been confirmed by many authors [8, 10, 12], this question still can not be regarded as conclusively settled. First, coordinated periodic activity of various parts of the alimentary tract was observed by V. N. Boldyrev even while food was present in the intestine. Investigators who have studied the periodic motor activity of the stomach have often had to remove a large amount of food from the stomach before an experiment, after which they could immediately record typical periodic contractions. These doubtless occur in the presence of a food mass in small intestine, i.e., under conditions of intensive absorption of food substances into the blood. For that reason, it is essentially incorrect to call periodic gastric contractions "hunger" movements. Secondly, several authors have observed periodic stomach contractions while food residues were present in the stomach cavity, i.e., at the end of gastric digestion [1, 6, 12]. Third, there are some indications that there is no perceptible difference between "hunger" movements and the digestive motor activity of the stomach [9]. In addition, some investigators [2, 5] have seen periodic release of bile from the gall bladder during gastric digestion. Up to two gall-bladder contractions in a 6-hour digestive period were observed in man. In dogs with fistulae of the bile duct it has been found that during the digestive process 2-4 gall-bladder contractions occur after giving milk or meat. The duration of contractions averages 40-80 min, and the duration of the intervals between contractions, when only liver bile is secreted, is 10-80 min. In studying the periodic motor activity of the stomach in dogs after chronic loss of bile, we also observed bile secretion of a periodic character both during digestion and on an empty stomach [7]. In these experiments we noted that when bile is eliminated from the digestive process over long periods of time, the weakening and disordering of the periodic motor activity of the stomach that results is paralleled by a weakening of the emptying function of the stomach. In this connection it is of particular interest to compare Pavlov's conclusion as to the emptying, "throwing-out" character of the movements of the gall bladder, which at that time had not yet been described as periodic [4].

The present paper is a report on experiments concerned with periodic motor activity of the stomach during digestion. The experiments fall into three series: 1) period motor activity of the stomach after removal of chyme through a gastric fistula and rinsing the stomach with warm water; 2) periodic motor actitivy of the stomach with food residues present in it; 3) periodic motor activity of the stomach when the dog was fed mixed food during the experiment.

METHODS

We conducted 46 experiments on six full-grown dogs in which the motor activity of the stomach was recorded for 8-20 hr or more. For these experiments we used the following dogs: Marta (with a fistula at the boundry between the pyloric and fundic portions of the stomach and a gall-bladder fistula with ligation of the common bile duct); Sil'va (having a fistula in the gastric fundus; resection of the pyloric segment with retention of the pyloric sphincter and an end-to-end anastomosis between the fundic and remaining pyloric parts of the stomach); Khitryi (fistula in the gastric fundus, resection of the whole pyloric segment, posterior gastroenteroanastomosis, and gall-bladder fistula with ligated common bile duct); German (fistula at the gastric fundus, and an isolated Pavlov pouch); and Belka and Dzhul'ba (fistula at the boundary between the pyloric and fundic portions of the

stomach). The dogs were kept on a constant mixed diet; bread, meat, and oatmeal. The food was given once a day, usually at noon. In the case of some of the dogs, part of the bread and oatmeal was found to be still in the feeding tray on the following day.

The dogs looked healthy, and their weight remained the same throughout the period of these experiments. The gastric contractions were recorded with the aid of a rubber balloon with a volume of 15-20 ml, connected to a Marey tambour by rubber tubing filled with water and air. The contractions were recorded on kymographs with special clock mechanisms to rotate the drums at a perfectly uniform rate. Two kymographs were used: one made a complete revolution of the drum in six hours, and the other in eight hours. It was not difficult to remove the food from the stomach, on account of the wide lumen of the fistula (2 cm diameter). The stomach was rinsed for 4-7 min with water warmed to 38°. The presence of food in the stomach during the experiment and the ejecting character of the gastric contractions were estimated from the expulsion of chyme through a hole in the stopper placed in the fistula. From time to time the stopper was removed from the fistula and the presence of food in the stomach was checked. In those experiments that ended with food still in the stomach, the chyme was removed through the fistula and weighed. The length of the experiments enabled us to compare the motor function of the empty stomach with its motor activity during digestion.

RESULTS

Removing food from the stomach and rinsing it with water causes the tone of the gastric musculature to be elevated for 1-2 hr. But the periodic activity of the stomach in this case is not noticeably different from the typical periodic motor activity that occurs when all food passes into the intestine. Since various amounts of half-digested food (from 100 to 800 g) were removed from the stomach in these experiments, there is reason to suppose that clear-cut periodic motor activity of the stomach was occurring in the presence of active intestinal digestion.

In experiments where the stomach was not rinsed, 100-400 g of chyme was pushed out through the opening in the fistula stopped by the gastric contractions. At the end of some experiments, up to 200 g of chyme was extracted from the stomach. In the course of these experiments it became clear that the presence of food in the stomach has a marked effect on the motor activity of the stomach, without disturbing its periodicity. A distinctive feature of such "digestive" periods, as compared to so-called "hunger" periods, is that they are thoroughly saturated with contractions during the phase of relative rest. In the empty stomach, after the end of a period of activity there follows a distinct period of rest for the gastric musculature for 1-2 hours, but during gastric digestion the resting phase is completely replaced by stomach contractions. The latter gradually become stronger and pass directly into contractions of the active period.

Figure 1 shows that the periodic changes in tone and the periodic development of strong contractions characteristic for the empty stomach (second half of the experiment) are also observed when food residues are present in the stomach (first half of the experiment). It can be seen from Fig. 1 that the clear-cut division of the motor activity of the stomach into periods of work and rest, which characterized the empty stomach of the dog, is largely masked during gastric digestion by contractions in the "resting" phase. In addition, we note some variability in the length of the "digestive" cycles which is less characteristic of the periodic activity of the empty stomach. Whereas the length of the "hunger" cycles was 1½ hr, on the average, that of the "digestive" cycles varied during a single experiment from an hour and twenty minutes to two hours and ten minutes. The periods of activity during gastric digestion were generally far longer than those of the empty stomach; the former usually lasted about an hour, in which time up to fifty contractions occurred. It was also noted that an individual periodic contraction, either during digestion or while the stomach is empty, is a complex peristaltic wave consisting of several component movements. Although contractions occur continually in the period of "rest" during digestion, the expression of food through the opening in

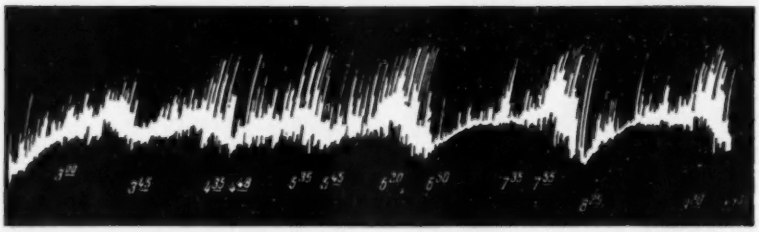


Fig. 1. Comparison of the periodic motor activity of the stomach when food is present in the stomach (first half of the experiment, up to 6 hours 50 minutes) and when the chyme has all moved into the intestine (second half of the experiment). Dog German. Experiment of November 2, 1952.

the fistula stopper occurs primarily during the period of activity.

Experiments with food present in the stomach also showed that the characteristics of the motor activity during digestion are similar to those of the empty stomach.

Thus, in one of the dogs the periodic motor activity of the empty stomach was irregular. During digestion the periodic motor activity of this dog's stomach had the same characteristics.

In experiments in which the dogs were fed mixed food during periodic activity of the empty stomach, the same results were obtained, in the main, as in those experiments where food remained in the stomach (Fig. 2). The act of eating itself, during the period of gastric "rest", brings on small contractions lasting 10-40 min. The elevation of "tone", which was almost always seen under these circumstances, was probably caused by mechanical pressure of the food on the balloon. The stomach contractions began during the very first minute of eating, and lasted for 5-7 min. The entrance of food into the stomach was accompanied by periodic motor activity, which lasted from five to ten hours, until all the food had moved into the intestine. Periodic motor activity then continued in the empty stomach. The first period of work during digestion came 1-3 hours after eating. During this 1-3 hours the stomach gave continual weak contractions, which gradually became stronger toward the beginning of the period of work. In the case where the dog ate 300 g of food (150 g of cooked meat and 150 g of thin oatmeal), the first period of work began 3-4 hr after the meal; it continued about an hour and was sometimes the only period of gastric work during the time of digestion. If the dog ate 600 g of food (150 g of meat, 100 g of bread, and 350 g of thin oatmeal), the first period of work more often began an hour after eating, and during gastric digestion 3-5 periods of stomach work were noted.

Thus, our experiments showed that during intestinal and gastric digestion periodic motor activity of the stomach occurs in dogs. There is no essential difference between periodic motor activity of the stomach during digestion and in the absence of gastric contents. A distinctive feature of the periodic motor activity of the stomach during digestion is a work period that is more prolonged than with the empty stomach, and a less marked period of relative rest.

The conditions that have permitted us to observe distinct periodic motor activity in the stomach during digestion are apparently the relatively long duration of our experiments under continuous-recording conditions, the method of slow recording of gastric activity, the maintenance of the dogs on a mixed diet, and the feeding of a mixture of foods during the experiment. These conditions distinguish our experiments from earlier ones.

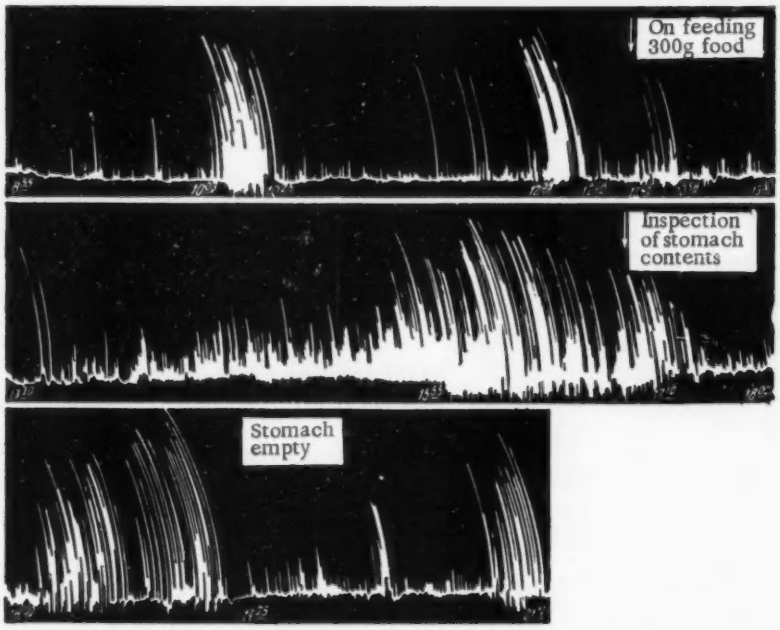


Fig. 2. The effect of eating a mixed meal on the periodic motor activity of the stomach. Small degree of excitation of gastric contractions in connection with eating. Three hours after meal, development of strong, prolonged period of gastric work, during which not all the food entered the intestine. After a second, also prolonged, period of work the stomach was free of food. Dog Dzhul'ba. Experiment of May 11, 1952.

In addition, our experiments give us reason to suppose that periodic motor activity of the stomach probably plays an important role in the natural transfer of food from the stomach into the intestine.

SUMMARY

A method of prolonged constant recording of the periodic motor activity of the stomach was used in chronic experiments on dogs with fistulae of various portions of the digestive tract. The existence of periodic gastric motor activity during intestinal and gastric digestion was revealed. This type of motor activity was recorded in dogs on mixed diets; these dogs also received a mixture of foods during the experiment. On the basis of these experiments it is suggested that periodic motor activity plays an important role in the natural passage of food from the stomach into the intestine. Stimulation of gastric motor function for 10-40 minutes in connection with the act of eating was also noted.

LITERATURE CITED

1. V. N. Boldyrev, Dissertation: Periodic Activity of the Digestive Apparatus in the Presence of an Empty Stomach [in Russian] (St. Petersburg, 1904).
2. E. F. Larin, Byull. Éksp. Biol. Med. 24, 10,315 (1947).
3. S. L. Lintvarev, Dissertation: The Role of Fats in the Transfer of the Stomach Contents into the Intestines [in Russian] (St. Petersburg, 1901).
4. I. P. Pavlov, Complete Collected Works [in Russian] (Moscow and Leningrad, 1951) Vol. 2, Book 2, p.261.
5. F. F. Saks and V. D. Sukhodolo, Transactions of the All-Union Society of Physiologists, Biochemists, and Pharmacologists [in Russian] (Moscow, 1952) Vol. 1, p. 94.
6. M. A. Sobakin, Byull. Éksp. Biol. Med. 28, 1, 48 (1949).
7. V. G. Startsev, Abstracts of the Twelfth Scientific Conference of Tomsk Medical Institute [in Russian] (1953) p. 20.
8. Z. D. Frumin, Fiziol. Zhur. 28, 5, 553 (1940).
9. E. T. Khrutskii, Fiziol. Zhur. 23, 2, 329 (1937).
10. I. P. Chukichev, The Problem of Protein in Physiology [in Russian] (Moscow, 1935) p. 37.
11. P. O. Shirokikh, Transactions of the Eleventh Congress of Russian Naturalists and Physicians [in Russian] (St. Petersburg, 1901) No. 10, p. 488.
12. T. L. Patterson, Ann. New York Acad. Sci. 34, 55 (1932).

CHANGE OF THE FUNCTIONAL STATE OF THE PERIPHERAL NEUROMUSCULAR APPARATUS DURING THE FORMATION OF A DOMINANT IN THE SPINAL CORD

Communication I. Influence of Spinal Cord Dominant on the Parameters of Excitability of the Nerve and Muscle

F. D. Sheikhon and G. V. Nikishin

Laboratory of Electrophysiology (Head-Doctor of Biological Sciences, O. V. Verzilova)
Institute of Normal and Pathological Physiology (Director-Active Member
AMN SSSR, V. N. Chernigovskii) AMN SSSR, Moscow

(Presented by V. N. Chernigovskii, Active Member, AMN SSSR)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 10, pp. 16-23, October, 1960
Original article submitted September, 1959

In their studies of the dominant, Ukhtomskii's school paid particular attention to the laws of the reorganization of intracentral relationship [5-8, 12, 13]. Only the work of Aristova [1, 2], from Vasilev's laboratory, was concerned with the question of the influence of the dominant center on the peripheral neuromuscular apparatus connected with such a center.

It was determined by one investigator [11] that a prolonged cooling of the frog, which led to the formation of a natural flexor dominant, was accompanied in the majority of cases by a change in the ratios of the chronaxies of the antagonistic muscles of the hind limb. If the frog were maintained at room temperature, the values for chronaxies of the extensor muscles were greater than those of the flexors, and the relationship of these values to each other was close to 2. According to the data of this worker, when the frog was kept at a low temperature, this relationship - the coefficient of Bourguignon - became equalized or less than unity, as a result of the rise of chronaxie of the flexors and the lowering of chronaxie of the extensor muscles. In the experiments of Aristova, rheobase and chronaxie changed in opposite directions. Unfortunately, this author recorded the rheobase and chronaxie of the muscles of only one extremity and did not examine the excitability of the motor nerve.

It thus remained unclear from the experiments of Aristova whether the dominant produced by cooling, which was reflected in the results obtained, was due to the presence of a dominant in both flexor centers for the hind limbs or in one of them only. Furthermore, the experimental arrangement was such as to preclude the possibility of following the dynamics of the changes in excitability of the muscles during formation and extinction of the dominant in one and the same animal, for the author compared the values of Bourguignon's coefficient in normal and preliminarily-cooled animals.

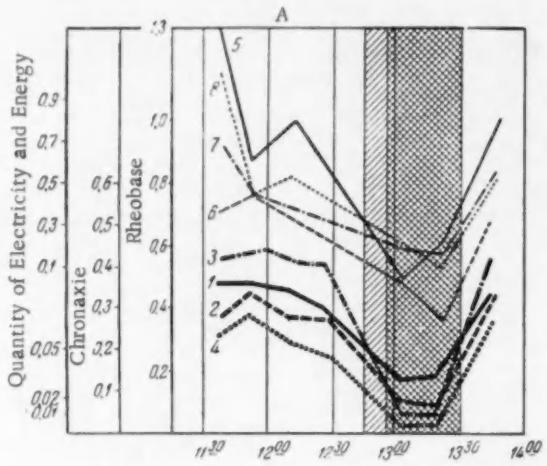
In our work, we set ourselves the task of studying changes of the functional state of the peripheral neuromuscular apparatus in the process of the appearance, development and extinction of an experimental, artificially produced dominant.

The dominant was produced by the action of rhythmic subliminal stimuli to the tibial nerve of the frog, according to the method of Vetyukov [4, 5], as modified by Verzilova [3]. The presence of a dominant was shown in the perversion of the reciprocal relations among the centers for the antagonists, which was expressed as a heightened contraction of the semitendinosus muscle to test stimulations of the nerves.

METHODS

The cerebral hemispheres of frogs Rana ridibunda were ablated and on both sides the semitendinosus muscles, peroneal nerves, ulnar nerves, and the motor branches of the sciatic nerve (deep posterior twigs) which innervate the semitendinosus muscles were prepared. The frogs were fastened to a cork board and placed in a moist chamber. Induction currents at a frequency of 50 cps and of an intensity 2-3 cm below threshold were used for subliminal excitation. These stimuli lasted for 10-20 min and generally were applied repeatedly. In the intervals between the action of subliminal stimulations reflex contractions of the semitendinosus muscles were recorded, against the background of which test stimuli were applied to the ulnar and contralateral peroneal nerves. The rheobase and chronaxie of the motor nerve (deep posterior branch of the sciatic nerve) and of the semitendinosus muscle innervated by it served as the characteristic of the functional state of the peripheral neuromuscular apparatus.

Silver chloride electrodes were used. Rheobase and chronaxie of the muscle were determined by means of



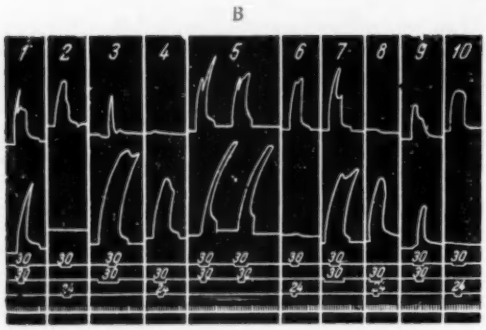


Fig. 1. Change of parameters of excitability of the motor nerve and muscle during formation of a dominant focus in the ipsilateral flexor center of the spinal cord. Experiment 34, April 9, 1957.

A. Reduction of rheobase, chronaxie, threshold quantity of electricity, and threshold energy of stimulating current of the semitendinosus muscle and its motor nerve during formation of a dominant in the flexor centers of the corresponding limb. The ipsilateral peroneal nerve was subjected to subliminal tetanization for 10 min (12:50 to 13:00, first hatched column). As a result of this a dominant arose (second hatched column).

1. Rheobase of the nerve; 2) chronaxie of the nerve; 3) threshold quantity of electricity of the stimulating current for the nerve; 4) threshold energy of the stimulating current for the nerve; 5) rheobase of the muscle; 6) chronaxie of the muscle; 7) threshold quantity of electricity of the stimulating current for the muscle; 8) threshold energy of the stimulating current for the muscle.

B. Kymograms from same experiment.

1st-4th sequences, reflex contractions of semitendinosus muscle at start of experiment, before formation of dominant; 5th-8th sequences, the same, after formation of dominant in the ipsilateral flexor center of the spinal cord due to influence of subliminal stimulation of peroneal nerve (frequency, 50 cycles; strength, 2 cm below threshold); 9th and 10th sequences, the same, after extinction of the dominant.

Significance of the curves (from above downward): myogram of the reflex contractions of ipsilateral semitendinosus muscle; myogram of reflex contractions of contralateral semitendinosus muscle; signal marker for stimulation of ipsilateral peroneal nerve; signal for stimulation of contralateral peroneal nerve; signal for stimulation of ulnar nerve; time marks (1 sec). Numbers above stimulation markers; distance between coils of inductorium (in centimeters).

needle electrodes, stuck into the belly and tendon of the muscle and separated by a distance of 10 mm. Measurements of excitability were carried out repeatedly – at the beginning of the experiment, at the time of formation of the dominant, and after its extinction. On the basis of the data obtained the threshold quantity of electricity (V_t) and the threshold energy (V^2_t) of the stimulating current required for excitation of the nerve and muscle were calculated. In all, 160 experiments were carried out. In the first series of experiments rheobase and chronaxie of the nerve and muscle of one limb were studied. These same parameters for nerve and muscle of one hind limb and of the motor nerve of the opposite extremity were determined simultaneously in the second series of experiments.

RESULTS

The experiments showed that the formation of a dominant in the flexor centers of the spinal cord was accompanied by a precise change in the parameters of excitability of the nerve and muscle. During this process there is a decrease of rheobase of the motor nerve and the muscle of the hind limb innervated by it, on the side ipsilateral to the dominant. Chronaxie was shortened, and the liminal quantity of electricity and energy of the stimulating current was reduced. The parameters of excitability of the motor nerve changed most sharply. Thus, in experiments carried out in the winter months (December-February), the values for rheobase and chronaxie of the motor nerve were reduced by 2-10 times. This observation is in good agreement with the fact that the spinal flexor dominant is established best in the winter.

When the dominant was formed, excitability of the muscle was heightened to a lesser extent than that of its motor nerve. With a deepening of the dominant state of the center there was an accompanying greater expression of the reduction of the parameters of excitation, but a weakening, on the other hand, of their expansion. The data from experiment 34 (Fig. 1) may be given as an illustration of this. At the beginning of the experiment, before application of subliminal stimulation, reciprocal relationships took place: reflex contractions of the ipsilateral muscle were weakened during stimulation of the contralateral peroneal nerve and ulnar nerve (Fig. 1B, 1st and 2nd strips of the myogram). Contraction of the contralateral muscle was reduced during test stimulation of the ipsilateral nerve (Fig. 1B, 3rd and 4th strips of the myogram). A ten min subthreshold tetanization of the ipsilateral nerve then led to the formation of a flexor dominant in the spinal centers for this extremity. Now, as is evident from the myogram, stimulation of the contralateral, peroneal, and ulnar nerves (Fig. 1B, 5th and 6th strips of the myogram) evoked an augmentation of the contraction of the ipsilateral muscle instead of inhibiting it, while at the same time the inhibitory effect was preserved in the opposite limb (Fig. 1B, 6th-8th strips of the myogram.). The rheobase of the ipsilateral motor nerve was reduced by 2.5

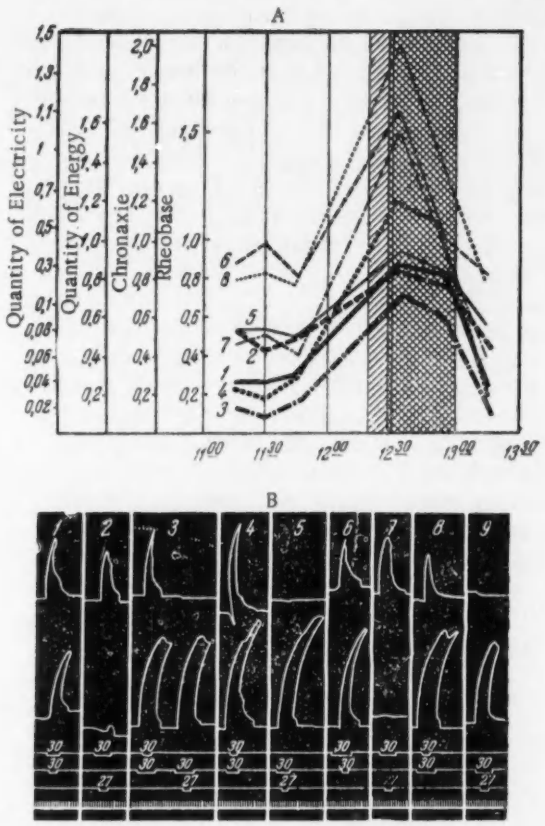


Fig. 2. Change of parameters of excitability of nerve and muscle during formation of a dominant focus in the contralateral flexor center of the spinal cord. Experiment 33, May 1, 1957.

A. Increase of rheobase, chronaxie, threshold quantity of electricity and threshold energy of stimulating current for semitendinosus muscle and its motor nerve during formation of dominant in flexor centers of the opposite limb.

The contralateral peroneal nerve was subjected to subliminal tetanization for 10 minutes (12:20 to 12:30, first hatched column). As a result of this a dominant was formed (second hatched column).

Designations on graph same as in Fig. 1A.

B. Kymograms of same experiment.

1st-3rd sequences, reflex contractions of semitendinosus muscle at beginning of experiment, before formation of dominant; 4th-7th sequences, the same, after establishment of the dominant under the influence of subthreshold stimulation of the contralateral peroneal nerve; 8th and 9th sequences, the same, after extinction of the dominant.

Significance of curves same as in Fig. 1B.

times (Fig. 1A, curve 1), and the chronaxie by 4 times (Fig. 1A, curve 2). In this experiment the threshold quantities of electricity (Fig. 1A, curve 3) and energy (Fig. 1A, curve 4) of the stimulating current were especially sharply reduced - by 10 and 25 times respectively.

Although less clearly expressed than the above, the parameters of excitability of the semitendinosus muscle also changed in the same direction during formation of the dominant. Rheobase of the muscle in this experiment was lowered by 2 times (Fig. 1A, curve 5), chronaxie by 1.6 times (Fig. 1A, curve 6), threshold quantity of electricity of the stimulating current by 2.5 times (Fig. 1A, curve 7), and threshold energy of the stimulating current by 5 times (Fig. 1A, curve 8). After extinction of the dominant the reciprocal relations between the centers of the muscle antagonists was again restored (Fig. 1B, 9th and 10th strips) and correspondingly reached the baseline levels of rheobase and chronaxie (see Fig. 1A).

Further experimentation showed that changes in the parameters of excitation of the motor nerve of the extremity contralateral to the flexor center in which the dominant was worked out were of an opposite character: rheobase increased, chronaxie lengthened, and threshold amount of electricity and threshold energy of the stimulating current increased.

Thus, for example, in experiment 33, of May 1, 1957, the rheobase of the nerve contralateral to the dominant of the extremity increased three-fold, chronaxie lengthened by 1.7 times, threshold quantity of electricity and energy of the stimulating current was augmented 5 and 15 5imes respectively (Fig 2A). Rheobase and chronaxie of the semitendinosus muscle change in the same direction.

These phenomena were demonstrated especially effectively in those experiments in which rheobase and chronaxie were measured in the nerve and muscle of an extremity in whose centers a dominant had been worked out, and in which the chronaxie of the motor nerve of the opposite limb was also studied at the same time (Fig. 3, experiment of May 11, 1957).

Changes in parameters of excitation are presented in Fig. 3. In each group pairs of columns show in sequence the changes of rheobase, chronaxie and threshold quantity of electricity and energy of the stimulating current. In each pair of columns the first shows the baseline value of the parameter before formation of the dominant, and the second, when the dominant is present. As is seen in Fig. 3, with the dominant formed in the ipsilateral flexor center, all parameters of excitation in this extremity were reduced. (Fig. 3A, hatched columns), whereas, on the contrary, they were increased on the opposite side (Fig. 3A, black columns).

When the dominant changed over from the ipsilateral to the contralateral flexor center, the opposite changes took place immediately (Fig. 3B). The parameters of excitability of the ipsilateral limb increased, whereas those of the contralateral side decreased. Such a transfer of the dominant from the flexor centers for one extremity to those of the other side often arose either spontaneously, or under the influence of repeated subliminal tetanization.

In some experiments subthreshold tetanization did not lead to the formation of a dominant in the spinal cord. When this occurred the values for rheobase, chronaxie, threshold quantity of electricity and of threshold energy of the stimulating current did not undergo significant changes. On the other hand, appearance of a "spontaneous" dominant (not dependent on subliminal tetanization) was accompanied by the characteristic reduction in the values for the indicated parameters.

The table above indicates changes of parameters of excitability of the nerve and muscle during formation of a dominant in the flexor center of one extremity. In 50 of the 64 cases reduction of the rheobase occurred and in 53 there was shortening of the chronaxie of the motor nerve of the ipsilateral limb. At the same time in 23 of a total of 34 experiments the rheobase increased and in 21 cases the chronaxie of the opposite side was lengthened. The parameters of excitation of the muscle also changed in the same direction during formation of the dominant. It should be exphasized that those experiments in which a dominant was formed simultaneously in the flexor centers for both limbs ("mixed dominant") are not entered in the data of this table. In such cases changes in excitability were of a more complicated character. They depended on the stage of expression of the dominant in one or another center. In some of these experiments we observed changes in rheobase and chronaxie which were in different directions.

The sum total of the data we have obtained leads to the conclusion that, during the formation of a dominant, important displacements can also be observed in the state of the peripheral neuromuscular apparatus along with those occurring in the reorganization of intracentral relationships (impairment of reciprocal inhibition). In such conditions excitability of the motor nerve, on the side of the dominant was increased, and was lowered on the opposite side. This also happened to a somewhat lesser degree with regard to excitability of the muscle.

Attention should be directed to displacements in the same direction of the parameters of rheobase and chronaxie at the time of appearance of the dominant - to their simultaneous shortening or lengthening, as the case may be. In contrast to Aristova's work changes of rheobase and chronaxie in opposite directions occurred in our experiments only in those cases where a mixed dominant was present. In various alterations of the nerve, rheobase and chronaxie often did change in opposite directions - chronaxie lengthened while rheobase decreased, and became shorter at a time when the rheobase was in-

Change of Rheobase and Chronaxie of a Motor Nerve and Muscle during Formation of a Dominant in the Spinal Cord

Parameters of excitability	On side of the dominant							On opposite side			
	Nerve				Muscle			Nerve			
	Number of cases			Character of change	Number of cases		Character of change	Number of cases			Character of change
	Total	of which			Total	of which		Total	of which		
Rheobase	64			Decrease	37		Decrease	34			Increase
		50	1	1:10		27	1	1:10	23	1	4:1
			3	1:5			1	1:4		3	3:1
			7	1:4—3			2	1:3		6	2:1
			14	1:2			3	1:2		7	1,5:1
			11	1:1,5			7	1:1,5		6	1—1,5:1
			14	1:1—1,5			13	1:1—1,5			No change
		7		No change		4		No change	6		Decrease
				Increase				Increase			
			2	2—3:1			1	2:1		1	1:1,5
		7	1	1,5:1		6	5	1:1—1,5	5	4	1:1—1,5
			4	1—1,5:1							
Chronaxie	64			Decrease	37		Decrease	34			Increase
		53	1	1:8		27	1	1:8	21	1	13:1
			3	1:4			1	1:4		3	3:1
			7	1:3			2	1:3		7	2:1
			14	1:2			4	1:2.5		6	1,5:1
			11	1:1,5			5	1:1.5		4	1—1,5:1
			17	1:1—1,5			14	1:1—1,5			
		3		No change		5		No change	5		No change
				Increase				Increase			Decrease
			2	3—2:1			2	2:1		1	1:3
		8	2	1,5:1		5	3	1,5:1	8	3	1:2
			4	1—1,5:1						4	1.1,5

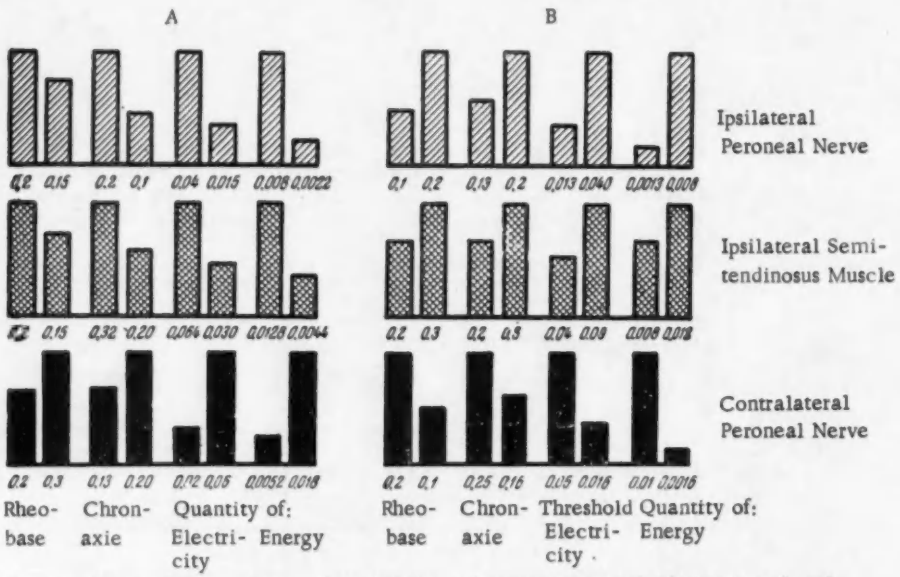


Fig. 3. Change of parameters of excitability of nerve and muscle during transfer of dominant from the flexor center for one limb to flexor center of the other side. Experiment 42, April 11, 1957.

A. Dominant in ipsilateral flexor center of spinal cord.

B. Dominant in contralateral flexor center of spinal cord.

In each group the pairs of columns designate, in order: rheobase, chronaxie, threshold quantity of electricity and threshold energy of the stimulating current. In each pair of columns, the first shows the baseline value of the parameter and the second, its value in the presence of the dominant. Figures under columns, the corresponding values of the parameters.

Legends: slanting hatched columns, parameters of excitability of the ipsilateral motor nerve; cross hatched columns, parameters of excitability of ipsilateral semitendinosus muscle; black columns, parameters of excitability of the contralateral motor nerve.

creased. On the basis of these facts Nasonov [9, 10] stated that the parameter of chronaxie was inversely dependent on the value of the rheobase and therefore could not be used as an indicator of the speed of rise of excitability in the nerve. In our experiments chronaxies shortened in spite of the fact that rheobase was reduced. It thus appears to us that this gives us a basis to consider that, during the rise of the dominant, a heightening of excitability (decrease of rheobase) takes place along with a decrease in the time of origin of excitation (chronaxie).

The changes which we have observed in the functional state of the neuromuscular apparatus during the working out of a dominant in the centers of the spinal cord may have an important adaptive significance, guaranteeing the preparation of an organ executing some task for the realization of an active effect. Further research is required for an understanding of the physiological mechanism of the action of the dominant center on the peripheral apparatus.

SUMMARY

The authors studied changes of the functional state of the peripheral neuromuscular apparatus during elaboration of the spinal cord dominant. The dominant was built up in one of the flexor centers of the frog's hindleg through application of rhythmic subliminal stimuli. The formation of the dominant manifested itself by inversion of the reciprocal relations between the centers of antagonists. It was found that, as the dominant appears in a flexor center of the spinal cord in a motor nerve and in the muscle innervated by it in the corresponding limb, rheobase, chronaxie, the threshold quantity of electricity, and the threshold energy of the stimulating current decrease, with simultaneous increase of all these parameters in the contralateral extremity. Disappearance of the dominant is accompanied by restoration of the initial level of excitability of the nerve and muscle, while the transfer of the dominant from a flexor center in one extremity to the centers of the other extremity

leads to corresponding modifications in their excitability.

LITERATURE CITED

1. N. K. Aristova, Influence of Dominant and Inhibitory Centers in the Central Nervous System on the Functional State of the Peripheral Neuromuscular Apparatus. Candidate's Thesis [in Russian] (Leningrad, 1953).
2. N. K. Aristova, Uchen. Zapiski LGU, Ser. Biol. 222, 106 (1957).
3. O. V. Verzilova, Byull. Éksp. Biol. Med. 45, 2, 12 (1958).*
4. I. A. Vetyukov, Transactions, Second All-Union Physiological Congress [in Russian] (Leningrad, 1926) p. 125.
5. I. A. Vetyukov, Selected Works from Physiological Laboratory, Leningrad State University, in Honor of 25 Years of Scientific Activity of A. A. Ukhtomskii [in Russian] (Moscow-Leningrad, 1930) p. 145.
6. M. I. Vinogradov, Fiziol. Zhur. 6, 47 (1923).
7. M. I. Vinogradov and G. P. Konradi, Mediko-biol. Zhur. 2, 63 (1928).
8. G. P. Konradi, Selected Works from Physiological Laboratory, Leningrad State University, in Honor of 25 Years of Scientific Activity of A. A. Ukhtomskii [in Russian] (Moscow-Leningrad, 1930) p. 118.
9. D. N. Nasonov and D. L. Rozental', Fiziol. Zhur. 39, 4, 405(1953).
10. D. N. Nasonov and D. L. Rozental', Fiziol. Zhur. 41, 1, 121 (1955).
11. Yu. M. Uflyand, Fiziol. Zhur. 8, 3-4, 132 (1925).
12. Yu. M. Uflyand, in: New Data in the Reflexology and Physiology of the Nervous System, Collection 1, [in Russian] (Leningrad-Moscow, 1925) p. 80.
13. A. A. Ukhtomskii, Collected Works [in Russian] (Leningrad, 1950) Vol. 1.
14. A. A. Ukhtomskii, Uchen. Zapiski LGU 22, 35 (1950).

* Original Russian pagination. See C. B. translation.

THE SPATIAL DISTRIBUTION OF ELECTRICAL ACTIVITY IN THE CEREBRAL CORTEX OF MAN AND ANIMALS

K. K. Monakhov

Laboratory of Electroencephalography (Head-Prof. M. N. Livanov)
of the Institute of Higher Nervous Activity (Dir. - Corresponding Member
AMN SSSR V. S. Rusinov) AN SSSR, Moscow
(Presented by Active Member AMN SSSR V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 10, pp. 23-28, October, 1960
Original article submitted October 26, 1959

The study of the bioelectrical activity of the cerebral cortex, which has attracted the attention of many investigators, has mainly followed the lines of study of parameters of frequency, amplitude, and so on in the electroencephalogram of individual points on the cortex. More general processes, encompassing the cerebral cortex as a whole, have received less attention, as has the spatial distribution of cortical activity.

In recent years there have been efforts to build instruments, called "toposcopes," that would give a spatial and topographic representation of the electrical activity of the cortex (Goldman, et al., 1948, 1949; Walter, et al., 1951; Shipton, 1952; Remond and Offner, 1952; Petsche and Marko, 1954, 1955; and Litty and Cherry, 1955).*

But the designs proposed have found only limited application in experimental work, and no practical application in the clinic. It is only through the invention of the electroencephaloscope and the appropriate methods [6] that it has become possible to employ toposcopy widely for the investigation of the bioelectric mosaic of the cortex and subcortical formations in clinical and experimental situations. This method permits us to study not only changes in the cortex as a whole, but also more localized and short-term processes. By drawing together the study of general and local processes in a single investigation, it gives more information about the regular features of the spatial distribution of the brain's electrical activity. This is especially important since the reflex mechanism, "which is the foundation of the central nervous system, comes down to a matter of spatial relationships" (I. P. Pavlov [9]).

The very first investigations involving the use of this new method disclosed a number of hitherto unknown regular patterns. These are concerned primarily with the appearance of foci of elevated activity during the establishment of a conditioned reflex, and with a particular form of synchronous electrical activity known as an "overflow," or spreading wave of activity [2, 5, 6, 7], the study of which is the subject of this paper.

METHODS

Examinations were carried out on persons whose cortical functional states were altered by the application of various influences: maximal deprivation of external stimuli, the development of sleep inhibition and return to the waking state, the influence of external stimuli, the production of a delayed conditioned reflex, performing an association experiment, etc. We also examined rabbits in states of waking and of sleep induced by amobarbital sodium. In this work we used an ÉÉS-50 electroencephaloscope (in an Anan'ev–Livanov system, 1955) and an electroencephalograph. The method of recording human cortical potentials, and the electrodes used, are described in an earlier paper of ours [8]. Here we will mention only that we distributed the electrodes evenly over the entire surface of the head, in five rows with 10 electrodes in each row. The cortical potential at each point was recorded in relation to the mean level of activity of the whole cortex.

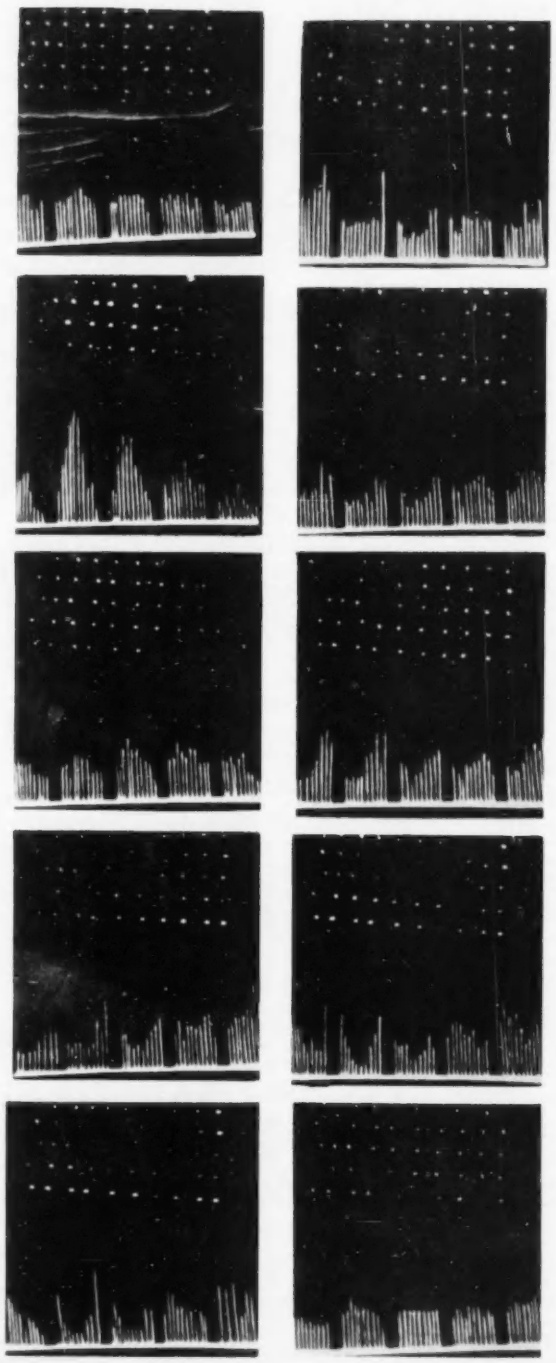
RESULTS

Our investigation showed that under certain conditions a distinctive change in electrical activity occurs in the cortical mosaic of healthy individuals and patients. That is, in one region of the cortex a synchronous elevation of electronegativity (from 30 to 200 μv) develops for a brief period (1/20-3/20 second), being displayed as an increase in the brightness of points on the electroencephaloscope screen. The next moment this electronegativity moves to a different region (lying either next to the first or some distance away from it) and thus, so to speak, executes motion, creating a spreading wave in a definite direction, leaving behind it a state of electropositivity. This motion may then occur in the opposite direction, or take a new direction (Fig. 1).

To obtain a clearer idea of the character of a spreading wave, we made a photometric analysis of a

*Cited by M. N. Livanov, V. M. Anan'ev, and N. P. Bekhtereva [7].

motion picture record containing a spreading wave, and described the activity of each point in numerical fashion. This numerical diagram shows how and within what range the activity changes during the passage of a spreading wave. From the figure presented herewith (Fig. 2) it can be seen that the electronegativity maximum in the parieto-occipital region moves to the frontal region (second frame). At this time electropositivity develops in the other parts of the cortex, with a maximum of its own and its own changes of position. The distinctive picture on the electroencephaloscope screen gives an idea of the form and the motion of the spreading wave.



The most obvious form of spreading wave that we have recorded was an elevation of electronegativity (positivity) in the frontal divisions corresponding to an electropositivity (negativity) in the occipital divisions. At the next instant this relationship was reversed. The accompanying figure (Fig. 2) shows a motion picture record in which the spreading waves described above are clearly seen. We call these "reciprocal" waves, since the relationship between the activity of the anterior and posterior portions of the brain at any given moment is a reciprocal one.

Along with the electroencephaloscopic study of this type of activity, we were interested in recording a spreading wave in electroencephalographic form. For this purpose we recorded cortical potentials from the same individuals under the same conditions by these two means.

Using bipolar recording with the electrodes located near the midline, about 2.5-3 cm apart (in the fronto-occipital direction), we recorded similar slow waves in all four leads. Examination of the phase of these variations disclosed that a shift occurred which amounted to a phase difference of 180° between the anterior and posterior regions of the brain. A calculation of the magnitude and direction of the phase shift coincided with the duration and direction of the spreading wave (Fig. 3, a).

Somewhat different relationships were observed with monopolar recording of the potentials. The four "active" electrodes were placed as in the preceding case; the "indifferent" electrode was placed on the ear lobe. As shown in Fig. 3, b and c, a difference in the amplitude of similar variations appears clearly in all four leads. A calculation of the magnitudes gave us an idea of the movement of the maximum amplitude over the cerebral cortex within the region where the electrodes were placed. On comparing the results with the electroencephaloscope

Fig. 1. A slow spreading wave with a complex "circular" motion, encompassing both hemispheres of the cerebral cortex (Successive frames of a motion picture record lasting 0.4 seconds. Each frame was exposed for 20 msec.). Explanation: in the upper part of the frame are 50 points, corresponding to 50 electrodes placed on the subject's head. The first row corresponds to the frontal electrodes, followed by fronto-parietal, parietal, and parieto-occipital; the last and lowest row corresponds to the occipital electrodes. The points on the extreme left and right correspond to temporal electrodes. In the bottom part of each frame the frequency-amplitude spectrum of all 50 points is recorded.

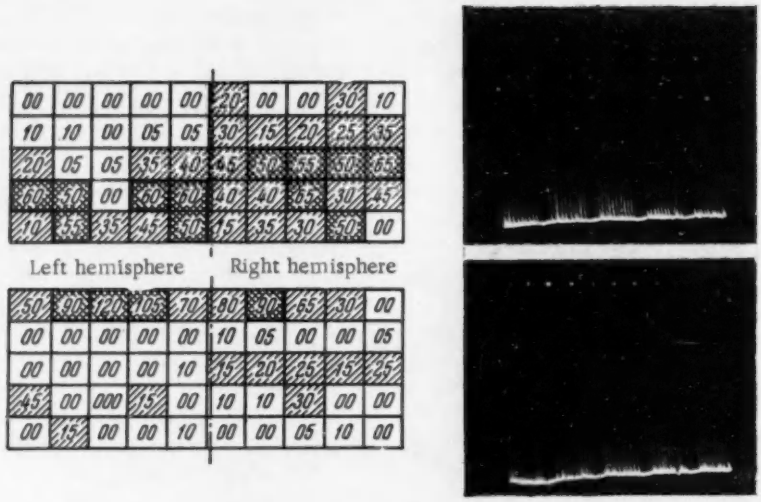


Fig. 2. Photometric analysis of a spreading wave (at the left is a numerical diagram of the wave represented in the photographs on the right). The explanation of the motion picture records is the same as in Fig. 1.

recording one is readily persuaded that this movement corresponds to a spreading wave. Simultaneous electroencephalographic and electroencephaloscopic recording established that there is complete correspondence between the spreading waves, with their recorded phase shifts, and the movement of the maximum amplitude of variations over the cortex.

The features described above can be understood on the basis of the concept that impulses which periodically reach the cerebral cortex and determine the state of local excitation within it strike different regions of the cortex at different times and with different intensities. For this reason the maximum electronegativity that develops at any given moment may move about over its surface.

The spreading wave as a form of bioelectrical activity can be characterized in at least four ways: first, with respect to the magnitude of the maximum of electronegativity (positivity); second, as to the time occupied by the passage of the spreading wave; third, with respect to the cortical area involved in the spreading wave – its "massiveness"; and finally, with respect to the direction of its movement and its tendency to recur.

In our investigations we succeeded in recording spreading waves lasting 1/10-1/12 sec, as well as longer ones lasting 1/3-1 sec. Two motion picture records can be cited as an example of the differences in duration (see Fig. 1 and Fig. 2). The first shows a wave lasting 400 msec, and the second shows a wave that occupied only 60 msec. Ordinarily the duration of the wave is related to the dominant rhythm in the electrical activity of the brain. The higher the frequency of potential variations, the faster the wave spreads, and vice versa. Figure 3 shows a spreading wave against a background of dominant alpha activity (b) and of slower delta activity (c). In the first case the amplitude maximum moves faster than in the second case. If the spreading wave repeats in sterotypic fashion, we may speak of its frequency, and the duration of the spreading wave can then be compared with the period of the dominant rhythm. It may equal the period of the dominant frequency or exceed it. With respect to the "massiveness" of the spreading waves one can say that this depends on the particular functional state. In our investigations the more extensive waves developed during the more profound states of sleep. During extensive spreading the extent to which the waves were directed was greater, and their pattern was repeated in a more stereotyped manner.

Consequently, on the basis of everything we have said it may be concluded that the spreading wave is a particular form of dynamic distribution of synchronous electrical activity in the cerebral cortex, in which the peak of electronegativity (positivity) moves from some cortical regions to others. In some instances the new regions are immediately adjacent to the first, while in others they are considerably removed. In this case a distinctive dynamic pattern appears on the electroencephaloscope screen, either one that is constantly changing or one that repeats itself in stereotypic fashion. The spreading wave is not connected with any one frequency of potential variations, but appears in its most obvious form when generalized slow rhythms are predominant in the cortex.

Some conditions favor the appearance or the enhancement of spreading waves. They may be recorded with particular clarity while the subject is asleep, when they are characterized by relatively slow movement in

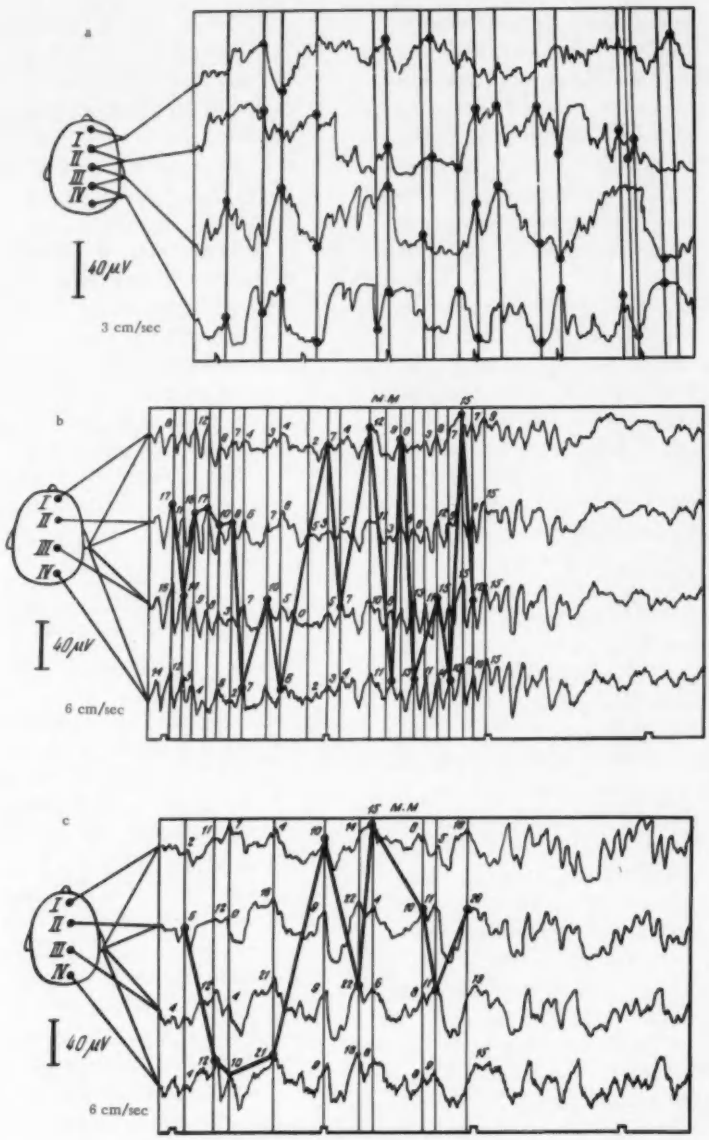


Fig. 3. Electroencephalographic form of a spreading wave recorded with bipolar (a) and monopolar (b and c) electrodes.

the fronto-occipital direction and, to a lesser extent, across the hemispheres. As a comparison with an electroencephalogram during sleep snowed, these slow spreading waves correspond to the delta wave stage. They usually disappear on arousal. But we have made some observations in which spreading waves were not present in the cortical mosaic during sleep, but reappeared shortly after awakening. Spreading waves can be recorded in the resting-waking state in the absence of external stimuli. In contrast to the spreading waves seen during sleep, these have a greater rate of movement and no definite directional tendencies, i.e., they move in various directions. The effects of external stimuli on spreading waves are different, depending on the initial functional state. Most often, application of an external stimulus (light, sound) against a background of spreading waves causes them to stop. On the other hand, when the same stimuli are applied during the waking state with no spreading waves present, they may appear in a pronounced degree. Apparently we must look for the cause of this phenomenon in the physiological intensity of the stimulus that evokes this reaction. A number of other conditions may also elicit the appearance of spreading waves. Thus, for example, the

development of a conditioned delayed motor reflex in man, and so-called conflict words in an association experiment, result in the appearance of this form of electrical activity. Marked spreading waves can be recorded in certain pathological brain conditions. N. P. Bekhtereva [1] has observed spreading waves in subcortical tumors of the brain. F. A. Leibovich [4] mentions stable transhemispheric spreading waves in epilepsy. We have observed spreading waves at the site of localization of arachnoiditis.

In investigations on rabbits it has also been observed that certain conditions favor the appearance of spreading waves. In particular, they appear in drug-induced sleep, and are the more pronounced the greater the dosage of amobarbital sodium. According to the data of I. N. Knipst, T. A. Korol'kova, and M. N. Livanov [3] spreading waves are also intensified in the stage of synchronization during the extinction of a conditioned reflex, and disappear when the conditioned reflex is reestablished. The effect of external stimuli on spreading waves in rabbits is similar to that in man.

Thus, the spreading wave is a rather widespread phenomenon in the electrical activity of the cerebral cortex of man and animals. Its mechanism is still unclear, but there have been suggestions that subcortical influences are important [1]. We also feel that the mechanism of spreading waves is connected with the activity of subcortical formations, but would like to underscore the significance of a definite functional state of the cortex for its realization. Interesting data have recently been obtained by our graduate student Yu. Izosimov, which suggest that the reticular formation of the midbrain is important for the appearance of spreading waves. Further study of this type of activity is of great interest, since a spreading wave, which usually appears against a background of intensification of inhibition in the cortex (sleep, extinction of conditioned reflexes, delayed inhibition, etc.) may be an indicator of the intensity of this process. Apparently, it may also serve as an index of the influence of the reticular formation on cortical tone.

SUMMARY

Using the electroencephaloscopic method of Livanov and Anan'ev, the author studied the spatial distribution of cortical electrical activity in man and animals. He noted that in certain physiological and pathological conditions of the central nervous system a special form of dynamic distribution of synchronous electrical activity (cortical mosaic) is recorded, called an "overflow," or spreading wave of activity, representing a movement of the peak of negativity (or positivity) from certain areas of the cortex into others, with a generalized rhythm of bioelectric activity all over the cortex. Recorded with monopolar leads, spreading waves appear as movement of the point of maximum amplitude of corresponding potential variations. With bipolar leads, it is manifested mainly as a phase shift in space.

Certain conditions for these spreading waves are described, and an attempt is made to analyze their mechanism.

LITERATURE CITED

1. N. P. Bekhtereva, Paper Presented to the Electrophysiological Seminar [in Russian] (Moscow, 1958).
2. N. A. Gavrilova, Zhur. Nevropatol. i Psikhiat. 59, No. 8 (1959).
3. I. N. Knipst, T. A. Korol'kova, and M. N. Livanov, Paper Presented to the Ninth All-Union Congress of Physiologists, Biochemists, and Pharmacologists [in Russian] (Minsk, 1959).
4. F.A.Leibovich,Zhur.Nevropatol.i Psikhiat. (in press).
5. M. N. Livanov, "On the Closure of the Conditioned Connection," International Colloquium on the Electroencephalograph of Higher Nervous Activity [in Russian] (Moscow, 1958).
6. M. N. Livanov and V. M. Anan'ev, Fiziol. Zhur. 4 (1955).
7. M. N. Livanov, V. M. Anan'ev, and N. P. Bekhtereva, Zhur. Nevropatol. i Psikhiat. 56, No. 10 (1956).
8. K. K. Monakhov and V. L. D'yakonov, Zhur. Nevropatol. i Psikhiat. 59, No. 8 (1959).
9. I. P. Pavlov, Collected Works [in Russian] (1951) Vol. III, Part I, p. 207.

THE EFFECT OF HYPOXIC HYPOXIA AND LOW BAROMETRIC PRESSURE ON THE HUMAN ELECTROCARDIOGRAM (Vector analysis)

V. N. Alifanov

Central Clinical Hospital (Director I. M. Pishugin)
USSR Civilian Airways, Moscow
(Presented by Active Member AMN SSSR V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 29-33, October, 1960
Original article submitted July 2, 1959

Most Russian authors who have studied the effect of low partial oxygen pressure on the human electrocardiogram (ECG) [1, 3-7, and others] have observed a reduction in the P and T waves. There is no one opinion agreed upon concerning the other waves. Similar results have been obtained by foreign authors [8, 10, 13, 14 and others]. G. V. Altukhov and V. B. Malkin [1] and other Russian authors have described the effect of low barometric pressure. They found that in a rarefied atmosphere corresponding to a height of 8,000-12,000 m, there is a rotation of the electrical axis of the heart (a change of the angle alpha) to the left due to the more horizontal position of the heart produced by a more elevated position of the diaphragm.

We have found accounts of only a few experiments in which vector methods of ECG analysis have been applied to traces recorded in hypoxia or at low barometric pressures. Thus, S. Tittel [14], and Gürtler, Poulsen, and Rasmussen [11] found that at low partial oxygen pressures, the integrative depolarization vector of the myocardium (AQRS) is reduced during inspiration, while maintaining its original direction; the repolarization vector (AT) is deviated to the left, but remains unchanged in amplitude; the "ventricular gradient" (G) changes both in direction and magnitude.

H. Becker-Freyseng and M. Stamberger [9] have described similar changes in the AT vector to be caused by exposure to reduced atmospheric pressure at a "height" of 11,500 m when breathing oxygen.

The vector method of ECG analysis is widely used in clinical practice. In attempting to reveal functional myocardial changes in human subjects caused by variations in the external factors already described, in addition to other methods we have used vector analysis of the electrocardiograms recorded at reduced atmospheric pressure.

METHOD

The experiments were carried out in a pressure chamber in which the pressure was reduced to 405 mm mercury, corresponding to a height of 5,000 m, and the subjects breathed the air of the chamber for 30 min. In investigating the effect of the very lowest pressure, we reduced it to 195-170 mm mercury, corresponding to a height of 10,000-11,000 m; the subjects breathed oxygen for 20-25 min. The ECG was recorded for 15-20 min before "ascending," at the 3-5th and 20-25th min after the particular "height" had been reached, and 3-5 min after "coming down." The three standard leads were used in addition to chest leads CR_2, CR_4, and CR_5. Besides the normal ECG analysis, we also made measurements of the frontal vectors (vectors were constructed on the triple axis system).

In all, 120 investigations were made, 25 in hypoxia, and 95 at reduced pressure.

Healthy male subjects aged 20-30 and showing no ECG anomalies were used. Strictly controlled conditions of work, feeding, and rest were maintained.

RESULTS

At a "height" of 5,000 m, without additional oxygen, a moderate degree of hypoxic hypoxia was well tolerated, and the ECG changes were as follows: the P_1 wave was usually increased, and P_{2-3} reduced; the precordial vector AP showed some tendency to rotate to the left, and it was usually reduced in magnitude; the P-Q interval and the Q and S waves showed changes which were not consistent. Quite frequently a left type of ECG was found, in which the R_1 wave was somewhat increased and R_{2-3} reduced; the AQRS vector was displaced to the left. The T_{1-3} waves were reduced, and the AT vector both reduced and as a rule displaced to the left. The "ventricular gradient" G changed in the same way as did AQRS.

Thus, there was a combined rotation of the precordial and ventricular vectors to the left without them becoming separated, which indicated a changed position of the ECG. Quite frequently there were signs of increased electrical activity of the left ventricle.

A confirmation of these findings was supplied from other axonometric measurements. The R/T ratio showed

no increase, and the R/S ratio changed, but not consistently.

In analyzing the ECG, we have thought it advantageous to introduce the R/P ratio (AQRS/AP), which describes the direction in space of the potentials in the myocardium of the auricles and ventricles. When the hypoxia is well tolerated, the index shows some increase on account of a comparatively small rotation of the AP vector to the left.

When the hypoxia is less well tolerated (Fig. 1), the P_{2-3} waves are relatively increased; the AP vector is quite frequently rotated to the right, and increased. The R_{1-3} waves are reduced, and the AQRS vector, particularly at the end of the experiment, is frequently rotated to the right. These changes indicate an increased load on the right heart, as is confirmed by several x-ray findings [2, 12]. The effects may be attributed to a hypertonia in the lesser circulation occurring in hypoxia. Changes in the T waves under these conditions are similar to those described above, but are frequently primary ("ischemic") in nature. The R/T ratio quite frequently increased, and the R/P (AQRS/AP) index increases on account of rotation of AP to the right and signs of overload of the right auricle, though it may remain unchanged or even decrease if in addition there is an increased load on the right ventricle (rotation of the AQRS vector to the right). There is frequently a reduction in the White index, which indirectly confirms the ideas presented above. The Fogel'son-Chernogorov systolic index showed no essential change.

When a human subject was exposed only to a reduced pressure, being brought to a "height" of 10,000 m and given oxygen, the ECG changes were considerably more complex than would have been expected from published reports. When the low barometric pressure was well tolerated, there was some tendency to a leftward rotation of the AP vector. When the condition of the subjects deteriorated, quite frequently the precordial vector was increased and rotated to the right; this effect was particularly well shown after "descent", when the masking influence of the elevated position of the diaphragm was removed.

When the low pressure is well tolerated a $Q_1 - S_3$ complex occurs which is well known to be typical of the elevated position of the diaphragm. When the pressure is not well tolerated, an $S_1 - Q_3$ complex appears which is typical of a rotation of the heart to the right about its longitudinal axis; however, in the conditions described, this result did not occur frequently, and in our opinion it indicates an increased load on the right heart.

When "high altitude" has no adverse effects, typically there was an increase in the R_1 wave and a reduction in the R_{2-3} waves, i.e., the AQRS vector rotates to the left. In such cases, the amplitude of the vector quite frequently increased, which gave reason to suppose that there was some association of the levogram with the relative increase of load on the right ventricle. When "high altitude" produced adverse effects, the voltage of

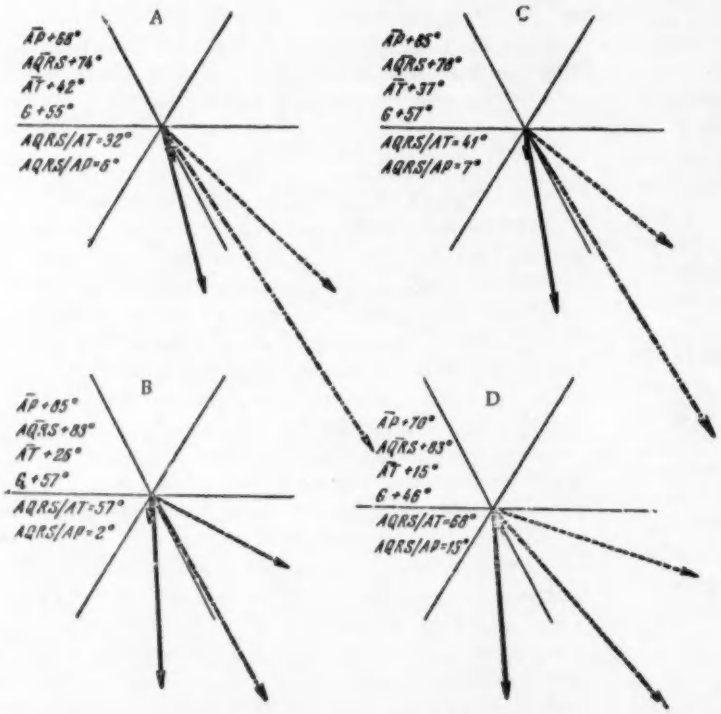


Fig. 1. Frontal vector cardiogram. Subject Ya-va, May 22, 1952. Hypoxia not well tolerated. A) Before "ascent"; B) 3 to 5 minutes at "high altitude; C) 20 to 25 minutes at "high altitude; D) after descent. AP – dotted line; AQRS – continuous arrow; AT – broken line; G – broken and dotted line.

the R_{1-3} waves was usually reduced; the AQRS vector quite frequently deviated to the right, particularly at the end of a period at "high altitude" which together with the right rotation of AP indicated that the predominant factor was the increased load on the right heart. Sometimes the ECG changes were typical of partial block of the right branch of the bundle of Hiss.

Changes in the T wave were less typical. The amplitude of the T_{1-3} wave was quite frequently reduced, the AT vector was deviated either to the left or to the right. The "ventricular gradient" at "high altitude" usually showed a rotation to the left. When "altitude" was reduced, the continuation of the "ventricular gradient" indicated the primary nature of the altered T waves. When the reduced pressure was well tolerated, the R/T ratio was reduced and the R/P (AQRS/AP) ratio quite frequently increased on account of the relative smaller rotation of AP to the left. When the condition of the subject deteriorated, there was a larger number of cases where the AQRS and AT vectors were separated in the frontal plane by more

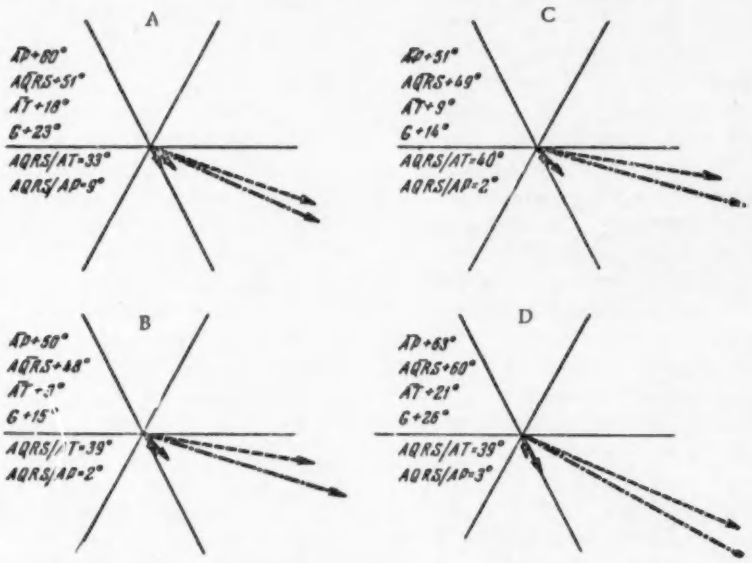


Fig. 2. Frontal vector cardiogram. Subject W-ov, February 12, 1952. Low pressure well tolerated. Indications as in Fig. 1.

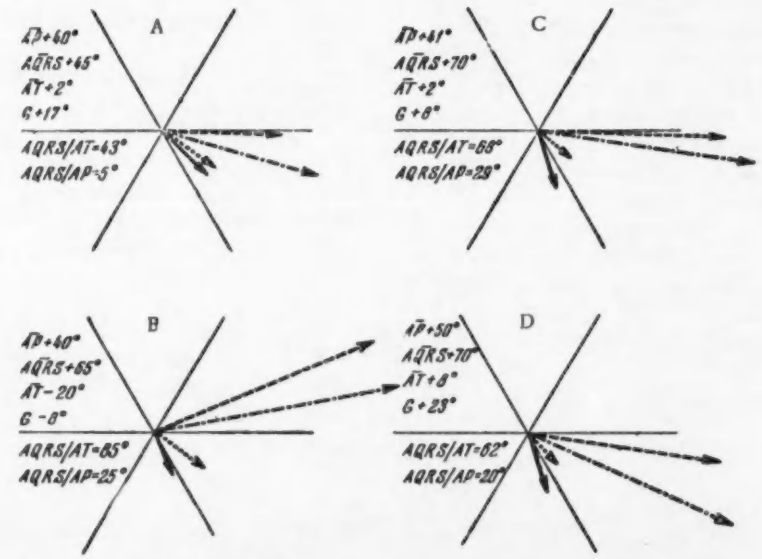


Fig. 3. Frontal vector cardiogram. Subject U-ov, February 14, 1952. Low tolerance to reduced pressure. Indications as in Fig. 1.

than 30°. The AQRS/ AP index was usually increased during the first few minutes at reduced pressure, but the change was due to the right rotation of AP combined with a preponderantly leftward rotation of AQRS. At the end of the period of "high altitude" the R/P ratio might be reduced on account of the additional right rotation of the AQRS vector.

The systolic index rose by 5-8% when the "altitude" was well tolerated; when the condition of the subject deteriorated, the index rose by 12-15%, indicating a disturbance of the ventricular myocardium (Figs. 2 and 3).

Changes of the ECG of the thoracic leads showed that when the reduced pressure was not well tolerated all the waves in lead CR_4 were reduced. In lead CR_2 the P, R, S, and T waves were increased; in lead CR_5 there was a moderate reduction in the S-T interval, i.e., there was a relative increase in the activity of the right heart.

It can be seen therefore that the appearance in the ECG of signs of overload of the right heart together with ischemia of one or other cardiac area (most frequently the left ventricle) seem to us to form a quite constant objective indication of a deterioration of condition following moderate degrees of hypoxia and reduced barometric pressure. Evidently, such changes may be ascribed to hindrance to the pulmonary circulation resulting from the hypertonia of the vessels of the lesser circulation induced by hypoxia, and also to abnormal interoceptive reflex influences brought about by an expansion of the hollow organs of the belly and some mechanical pressure on the lungs due to the elevation of the diaphragm ("high altitude meteorism").

SUMMARY

One hundred and twenty observations were made of the effect of a short stay in an altitude chamber on the ECG of healthy subjects at an effective "altitude" of 5,000 m while breathing either air, which induced hypoxia, or at an equivalent height of 10,000 m while breathing oxygen. In subjects who tolerated the hypoxia there was a combined swing to the left of the AP and AQRS-T vectors without any definite cardiac displacement. When the hypoxia was not well tolerated, after about 20-25 min at the reduced pressure there was a relative swing to the right of the cardiac vectors, indicating an increased load on the right heart. When no adverse effects resulted, the shift of the cardiac vectors to the left was accompanied by signs of increased left ventricular activity. When the subject's condition deteriorated, an ECG of the S_1-Q_3 type developed, and the AP and often the AQRS vector swung to the right while the R/T and R/P indices rose, which also indicated an increased load on the right heart. Therefore an ECG indicating such an increased right heart load constitutes also an objective sign of a deterioration of the physical condition.

LITERATURE CITED

1. G. V. Altukhov and V. B. Malkin, Klin. Med., 10, 45 (1952).
2. A. A. Gorodetskii, Transactions of the Conference on the Problem of Oxygen Insufficiency of the Organism [in Russian] (Kiev, 1949) p. 27.
3. M. A. Lyass, Transactions of the Central Military Academy of Electrolytic Apparatus, Civil Airfleet (1941) No. 1, p. 57.
4. O. P. Minut-Sorokhtina and N. V. Raeva, Fiziol. Zhur. 34, 2, 269 (1948).
5. V. G. Mirolyubov and I. A. Chernogorov, Klin. Med., 8, 1163 (1934).
6. N. S. Molchanov, Transactions of the Military Med-Academy [in Russian] (Leningrad, 1941) Vol. 31, p. 106.
7. V. A. Tsygankov, Transactions of the Military Med-Academy [in Russian] (Leningrad, 1940) Vol. 23, p. 77.
8. W. Adamaszek, Luftfahrmedizin Vol. 3, p. 125 (1939).
9. H. Becker-Freyseng and M. Stamberger, Jahrbuch der wissenschaftlichen Gesellschaft für Luftfahrt (Braunschweig, 1954) p. 184.
10. O. O. Benson, J. Aviation Med. Vol. 11, p. 67 (1940).
11. H. Gürtler, H. Poulsen, and K. N. Rasmussen, Acta med. scandinav. Vol. 150, p. 331 (1954).
12. H. Kottenhoff, Luftfahrmedizin Vol. 2, p. 194 (1938).
13. E. Opitz and O. Tilmann, Luftfahrmedizin Vol. 1, p. 153 (1936).
14. S. Tittel, Luftfahrmedizin Vol. 4, p.328 (1940).

RESPIRATORY ELECTROMYOGRAMS DURING RESISTANCE TO BREATHING BEFORE AND AFTER VAGAL SECTION

Yin Chi-chang

Laboratory of the Comparative Pathology of the Nervous System (Head-Prof. S. I. Frankshtein)
Institute of Normal and Pathological Physiology (Director-Active Member AMN SSSR V. N. Chernigovskii) AMN SSSR, Moscow
(Presented by Active Member AMN SSSR V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 34-40, October, 1960
Original article submitted October 21, 1959

A number of studies have been reported of the effect on ventilation, gaseous exchange, and respiratory movements by added resistance to breathing [1, 2, 5, 6, 9, 11, 22, 25, 27, 29, 37]. The respiratory movements were usually recorded pneumographically, and the work performed by the respiratory muscles was inferred only from their total effect. Nevertheless, it is known that respiratory disturbances may result from the impairment of individual muscles. The most convenient method for study has been electromyography (EMG).

The large amount of material on the EMG of the different respiratory muscles has accumulated since Dittler in 1909 first recorded potentials from the diaphragm. Some of the experiments were performed on animals [4, 7, 16, 19, 21, 30, 32, 36] and some on man [8, 13, 18, 24, 26, 31, 34].

There are only occasional references to the electrical activity of the respiratory muscles in man when breathing against a resistance. Campbell made a study of the potentials from the muscles of the abdomen. Normally, when surface electrodes are used, electrical activity from the muscles is not recorded. Campbell showed that it does not appear in these muscles when there is an added resistance. L. L. Shik [8] recorded the electrical activity of the intercostal muscles by means of surface electrodes and showed that it increases with added resistance to breathing.

We have made a simultaneous study of the electrical activity of the diaphragm, intercostal, and oblique abdominal muscles in experiments in which an added resistance to breathing was established chronically.

METHOD

The experiments were performed on 10 unanesthetized dogs weighing 9-17 kg. In the first series, resistance was added to the breathing of normal animals, and in the second series the vagi were divided. All the animals were taught to lie on their side on the table and to breathe through a mask. Potentials were recorded by means of electrodes sewn directly into the respiratory muscles [3]. Silver plate electrodes having an area of 10 x 6 mm were sewn to the left arch of the diaphragm and to the left oblique abdominal muscles through a slit in the peritoneum, the distance between the electrodes being 1.5 - 2 cm; smaller electrodes measuring 5 x 3 mm were attached to the intercartilaginous portion of the left internal intercostal muscles in the fifth intercostal space near the sternum at a separation of 1 cm. The potentials from these muscles were amplified in a three channel amplifer and recorded on a cathode ray oscillograph, the overall sensitivity being 30-100 μv/mm.

The resistance was added by a device which compelled the dog to breathe through a water column of a certain height. The preliminary results showed that there were considerable variations in the amount of resistance which each animal could tolerate. In our experiments therefore we did not use a standard value but one which was maximal for each animal, and which varied from 5 to 20 cm of water. In most experiments the pulmonary ventilation was measured by gas flowmeters. Muscle potentials and pulmonary ventilation were measured before and after introducing the added resistance. The experiment was repeated after dividing the vagi in the neck under local novocaine anesthesia.

RESULTS

Normal EMG of respiratory muscles. Before the added resistance (Fig. 1) the EMG of the diaphragm is asynchronous, gradually increasing at inspiration and decreasing at the end of expiration. With our method of recording, the maximum amplitude of the potential varied between 200 and 500 μv. At expiration the potential was smaller, lying between 50 and 300 μv. Diaphragm activity has previously been recorded both at expiration

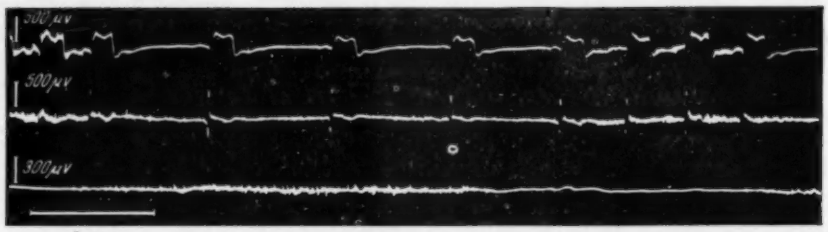


Fig. 1. Normal electromyogram of the respiratory muscles (dog Faithful, experiment of December 18, 1958). Curves (from top down): electromyogram of intercostal muscles, diaphragm, and oblique abdominal muscles.

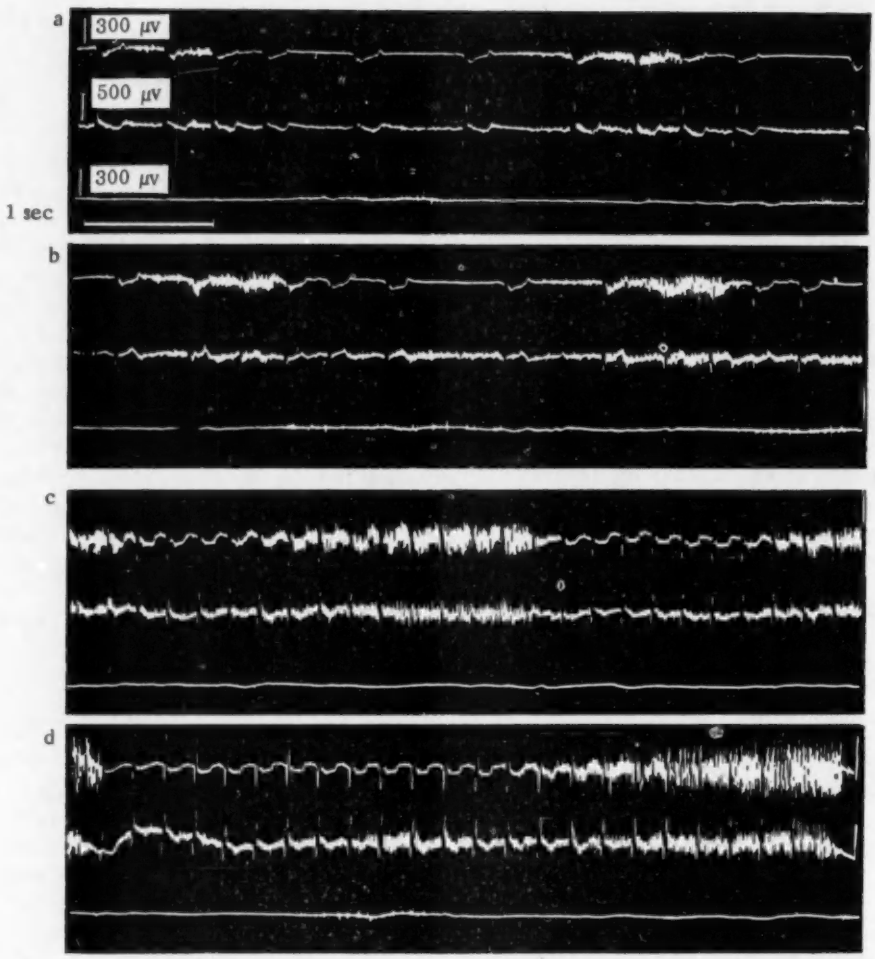


Fig. 2. Reaction of the respiratory muscles to a resistance to breathing before and after vagotomy (dog Faithful, experiment of March 23, 1959). Curves as in Fig. 1. a) Original background; b) after introducing resistance to breathing; c) after vagotomy, before increasing resistance; d) after increasing resistance.

and inspiration by other authors [16, 23, 31, 36]. These results correspond with the x-ray findings which indicate that the diaphragm does not relax completely at expiration.

The results on the electrical activity of the intercostal muscles obtained by previous workers are contradictory. The differences are evidently due to variation in the position of the electrodes. Nevertheless, it has been repeatedly shown that the potentials from the intercartilaginous portion of the internal intercostal muscles arise chiefly at expiration [10, 20, 30]. Our experiments have confirmed that the EMG of this part of the internal intercostal muscles typically shows impulses at inspiration and that they coincide with those from the diaphragm. Their amplitude varies between 120 and 400 μv.

Recordings from the internal oblique muscles showed that in the great majority of experiments there was an increase followed by a gradual decrease of electrical activity only at expiration, though occasionally there was an intermittent, but increasing, discharge during this phase. The maximum amplitude of the potentials was 50-250 μv.

Simultaneous recording from the different respiratory muscles showed that changes in diaphragm activity at expiration are closely associated with the changes in activity of the oblique muscles. The latter increases at expiration together with increased action from the diaphragm. Evidently, the activity of the diaphragm at expiration is brought about reflexly by its extension, prevents the sudden collapse of the lungs and brings about the normal smooth expulsion of the air. It is important to note that the oblique muscles first contract before the action of the diaphragm at inspiration has been completed. It appears that such an overlap enables a smooth change-over to be made between the inspirational and expirational phases [14].

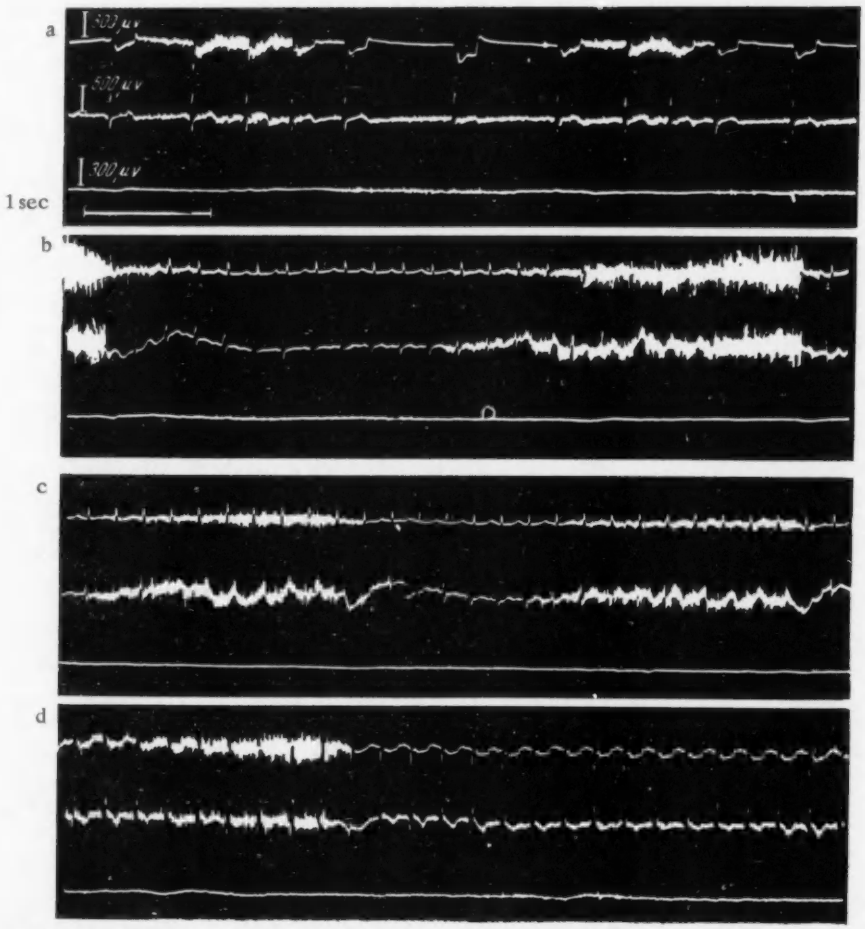


Fig. 3. Changes in the electrical activity of the respiratory muscles after vagotomy (dog Belka, experiment March 23, 1959). Curves as in Fig. 1. a) Before vagotomy; b) immediately after vagotomy; c) one hour after vagotomy; d) one day after vagotomy.

The EMG has shown that when there is a resistance to breathing, there is an increase of both inspiratory and expiratory muscle potentials, the amplitude increasing by 20-100% (Fig. 2a and b). However, despite the increased activity of the respiratory muscles, pulmonary ventilation was reduced by 5-15%.

EMG of the respiratory muscles after vagotomy. Before the increased resistance was introduced (Fig. 3), vagotomy by itself caused a marked change in the activity of the respiratory muscles. The discharge from the diaphragm and intercostal muscles after vagotomy showed a high rapidly increasing amplitude at inspiration, which immediately ceased when the breath was fully drawn, instead of relaxing gradually as in a normal animal. The amplitude of the potentials increased by as much as 2-6 times. Wachholder and McKinley [36] found a similar increase in the response of the diaphragm in vagotomized rabbits, and Meda and Ferroni [28] found the same effect in guinea pigs.

By contrast, the amplitude of the discharge from the internal abdominal muscles after vagotomy was markedly reduced, and often disappeared altogether. There was a corresponding reduction in amplitude or disappearance of the potentials from the diaphragm. The overlap of activities of the diaphragm and oblique muscles, which was commonly observed in normal animals, was not present after vagotomy.

Thus, the increased depth and slowing of the respiration occurring after vagotomy is associated with a marked increase and duration of the activity of the inspiratory muscles, while the activity of the muscles of expiration is reduced or absent. In addition, the respiration is less even.

Some time after vagotomy, the activity of the diaphragm and intercostal muscles at inspiration gradually became reduced, though it remained above normal. At the end of inspiration it broke off less sharply. The oblique muscles were active at inspiration a few hours after vagotomy, though sometimes not until two days afterwards, but the contractions did not have their full strength (see Fig. 3).

In vagotomized animals, added breathing resistance increased the amplitude of the potentials from the diaphragm and intercostal muscles at inspiration to a value 50-200% above the normal level (Fig. 2e and d).

When resistance to breathing was added, the electrical activity of the respiratory muscles was increased while pulmonary respiration was reduced, and this lack of correspondence was greater after vagotomy. Despite the marked increase in the amplitude of the potentials from the respiratory muscles, pulmonary ventilation decreased by 10-40%. By the second day after vagotomy, the increase in potentials from the respiratory muscles caused by increasing the resistance was not so well shown as on the first day (see table).

TABLE. Increase in the Amplitude of Impulses from the Diaphragm in Response to Added Breathing Resistance, on the same day as the Vagotomy, and two days after (in percent).

Name of dog	Amplitude of potentials from diaphragm	
	On the same day	On second day
Mushka	122.0	20.0
Volchok	150.0	80.0
Belka	66.6	13.3

It is known that normally respiratory movements show a characteristic gradual inspiration and expiration and a smooth transition between them. Our experiment showed that this effect is normally brought about by the gradual waxing and waning of the activity of the respiratory muscles, by the action of the diaphragm at expiration, and by the overlap of the periods of action of the diaphragm at inspiration and of the oblique muscles at expiration.

Division of the vagi seriously disturbs the coordination of respiratory movements. After vagotomy there is a marked increase in the activity of the diaphragm and intercostal muscles at inspiration, but it immediately breaks off at the end of this phase; during inspiration the action of the oblique abdominal muscles ends sharply; "during inspiration" lastly, there is no overlap between the inspirational and expirational phases.

It is known that most of the afferent impulses to the respiratory center from the lungs pass along the vagi. It might be thought that the failure of coordination of the respiratory muscles which has been described above might be attributed to disturbance of the respiratory center following its partial deafferentation by vagotomy. At a certain time after vagotomy has been performed, respiration becomes more or less even. Our records showed that this effect is brought about by the respiratory muscles ceasing their activity at input less sharply, and developing an action at expiration. The reason for this change is apparently that at a certain time after vagotomy there is an increase in the afferent inflow along other pathways such as the spinal nerves, sympathetic pulmonary nerves, and the sensory nerves of the pleura and of the respiratory muscles.

There are two views as to what is the mechanism of the change in the respiratory movements which follows increased resistance. Some authors consider that the explanation must be in terms of humoral regulation and particularly the accumulation of CO_2 in the blood [11, 25, 37]; others emphasize the importance of nervous regulation, and particularly the part played by the vagi [1, 8, 15, 17, 27, 35].

Our results favour the latter view. The EMG showed that resistance produced a greater change in vagotomized than in normal animals. The result was less well shown on the second day after vagotomy. It is known that the EMG of the respiratory muscles is an index of the condi-

tion of the respiratory center. It may therefore be supposed that the increased response to added resistance immediately after vagotomy is due to an increased excitability of the respiratory center brought about by the elimination of the principal afferent pathways. The reduction in the response on the second day after vagotomy is probably due to a reduction in the excitability of the respiratory center, which is caused by other afferent pathways being established.

Because the oblique muscles are no longer active after vagotomy, the restoration of their function in vagotomized animals when resistance is added indicates that the increased resistance acts as a further afferent stimulus to the respiratory center.

From what has been said, it follows that changes in the excitability of the respiratory center, which depend on an alteration in its afferent supply, play an important part in bringing about the functional changes in the respiratory musculature which are induced added resistance to respiration.

It has long been known that the increase in respiratory movements brought about by added resistance does not correspond to pulmonary ventilation. L. L. Shik and his co-workers found that in various pulmonary conditions ventilation was unchanged despite an increased activity of the respiratory muscles. The marked lack of correspondence between the increased activity of the respiratory muscles and the reduced ventilation which we observed to occur against an added resistance after vagotomy shows that the pulmonary ventilation depends not only on the activity of the respiratory muscles, but also on other factors associated with the vagi, such as control of the tone of the bronchials, innervation of the larynx, and the development of physiological atelectasis.

SUMMARY

Electrical activity of the respiratory muscles, including diaphragm, intercartilaginous part of the internal intercostal and internal oblique abdominal muscles, was recorded from electrodes chronically implanted in dogs. The disturbed coordination of the respiratory muscles which followed vagotomy was indicated by a sharp arrest of the increased electrical activity at the end of inspiration, and its failure to occur during expiration; in addition there was no longer any overlap between the activities in the inspiratory and expiratory phases. These alterations are attributed to functional changes occurring in the respiratory center and caused by the partial deafferentation through vagotomy. The functional changes in the respiratory musculature caused by introducing resistance to breathing depend on the excitation of the respiratory center, which in turn is determined by its afferentation.

LITERATURE CITED

1. B. A. Botvinnikov, I. Sh. Ginzburg, P. M. Gramenitskii, et al., in: Functions of the Organism Under Conditions of an Altered Gaseous Medium [in Russian] (Moscow, Leningrad 1955) No. 1, p. 118.
2. D. A. Bytchenko, Contribution to an Evaluation of the Type of Respiration with a Physical Load in a Gas Mask, Dissertation [in Russian] (Saratov, 1947).
3. T. I. Goryunova, Fiziol. Zhur. 44, 12, 1160 (1958).
4. T. I. Goryunova, Byull. Éksp. Biol. Med. 46, 9, 62 (1958).*
5. P. K. Isakov, Byull. Éksp. Biol. Med. 23, 3, 225 (1947).
6. A. M. Kulik, in: The Physiology and Pathology of Respiration, Hypoxia, and Oxygen Therapy [in Russian] (Kiev, 1958) p. 211.
7. E. S. Fedorchuk, The Effect of Stimulating the Carotid Labyrinth on the Electrical Activity of Skeletal Muscles. Dissertation [in Russian] (Leningrad, 1957).
8. L. L. Shik, in: The Physiology and Pathology of Respiration, Hypoxia, and Oxygen Therapy [in Russian] (Kiev, 1958) p. 108.
9. R. G. Bartlett, H. F. Brubach, R. C. Trimble, et al., J. Appl. Physiol. 13, 194 (1958).
10. R. C. Brown, A. K. Atkinson, and R. Gesell, Am. J. Physiol. 126, 447 (1939).
11. C. C. Cain and A. B. Otis, J. Aviation Med. 20, 149 (1949).
12. E. J. M. Campbell, J. Physiol. 136, 556, (1957).
13. E. J. M. Campbell, The Respiratory Muscles and the Mechanics of Breathing (London, 1958).
14. H. Sawazaki, Jap. J. Vet. Sci. 17, 6, 231 (1955).
15. H. W. Davies, G. R. Brow, and C. A. L. Binger, J. Exper. Med. 41, 37 (1925).
16. R. Dittler, Arch. Ges. Physiol. 130, 400 (1909).
17. A. Fleisch, Ergehn. d. Physiol. 36, 249 (1934).
18. W. F. Floyd and P. H. S. Silver, J. Anat. 84, 132 (1950).
19. R. Gesell, Am. J. Physiol 105, 37 (1933).
20. R. Gesell, Am J. Physiol. 115, 168 (1936).
21. R. Gesell, C. S. Magee, and J. W. Bricker, Am. J. Physiol. 128, 615 (1940).
22. F. G. Hall and F. Zechman, Proc. Soc. Exp. Biol. and Med. 96, 329 (1957).
23. P. Hoffmann, M. Schneider, and C. J. Keller, Z. Biol. 91, 196 (1931).
24. D. S. Jones, R. T. Beargie, and J. E. Pauly, Anat. Rec. 117, 17 (1953).
25. E. M. Killik, J. Physiol. 84, 162 (1935).

26. G. H. Koepke, A. J. Murphy, J. W. Rae, et al., Arch. Phys. Med. 36, 217 (1955).
27. Ya-Ko Lin and Hung-dar Hsu, Acta Physiol.sinica, 21, 1, 74, (1957).
28. E. Meda and A. Ferroni, Boll. Soc. ital. biol. sper. 32, 500 (1956).
29. R. L. Moore and C. A. L. Binger, J. Exper. Med 45, 1065 (1927).
30. Okabe, Acta paediat. japonica 62, 72,(1958).
31. P. H. Rossier., H. J. Nieporent., H. Pipberger., et al. Z. qes. exper. Med. 127, 39 (1956).
32. T. A. Sears., J. Physiol. 142, 350 (1958).
33. T. Shiratori, et al., Tohoku Jgaku Zassi 57, 412 (1958).
34. T. Tokizane, K. Kawamata, and H. Tokizane, Japan J. Physiol. 2, 232 (1952).
35. M. Uddstromer, Nasal Respiration (Helsingfors,1940) p. 50.
36. K. Wachholder and C. McKinley, Arch.ges. Physiol. 222, 575 (1929).
37. F. Zechman, F. G. Hall, and W. E. Hall, J. Appl. Physiol. 10, 356 (1957).

* Original Russian pagination. See C. B. translation.

THE EFFECT OF ARTIFICIAL PNEUMOTHORAX ON AFFERENT AND EFFERENT IMPULSES IN THE RESPIRATORY PATHWAYS

M. I. Vinogradova

Department of General Physiology (Head-Prof. A. V. Rikkl') Institute of Experimental Medicine (Director-Active Member AMN SSSR Prof. D. A. Biryukov) AMN SSSR, Leningrad
(Presented by Active Member AMN SSSR P. S. Kupalov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 40-45, October, 1960
Original article submitted July 23, 1959

Although artificial pneumothorax has long been used in clinical practice, the problem of its effect on the receptors and on the respiratory center is still far from clear. Nevertheless, a study of this problem would help to resolve certain points concerning the therapeutic effects of the operation.

One method of revealing changes in the activity of receptors or central neurones in response to various stimuli is to record the action potentials in the nerve trunk concerned. Of the electrical recordings from nerves which are known to us, only those of D. A. Kocherga [5] and V. M. Shirokaya [9 and 10] and some of those of Larrabel and Knowlton [14] deal with the effect of pneumothorax. There is no agreement between the results found by D. A. Kocherga and V. M. Shirokaya, who do not distinguish between the activity of the different receptors of the respiratory apparatus in artificial pneumothorax. The reason is that they picked up potentials from relatively thick bundles containing a large number of functionally distinct nerve fibers.

The present work represents a direct continuation of our previous investigations of the afferent and efferent impulses in the nerves of the respiratory apparatus [1, 2, 3].

METHOD

We led off the action potentials, from thin bundles separated from the vagi in the neck, or, when studying efferent impulses, from its recurrent laryngeal branch. The small number of fibers present in these bundles makes it possible to carry out a more detailed study of the response of the receptors to the artificial pneumothorax. The work was carried out on cats and rabbits anesthetized with thiopental sodium. The potentials were recorded by means of a cathode ray oscillograph, and at the same time pressure variations in the intrapleural pressure were recorded on a motion-picture film, and in some of the experiments a record was made of the pressure in the carotid or femoral artery.

RESULTS

As can be seen from the oscillogram of one typical experiment (Fig. 1, A) the introduction of 10 ml per kg of air into the pleural cavity reduced the frequency of the impulses from the pulmonary stretch receptors at inspiration. The effect evidently arises because the lung is less inflated. The frequency remains reduced for 10-15 min, and then immediately returns to its original value when air is sucked out of the pleural cavity (Fig. 1, A, c). If large amounts of air up to 17 ml per kg are introduced, the reduction in the rate of afferent impulses is greater (Fig. 1, A, d).

When the results of the electrophysiological experiment were compared with a dissection made subsequently, it was found that in animals in which the mediastinum was firm there was no change in the frequency of nerve impulses on the side opposite to that on which the pneumothorax had been established, but in those in which it was thin and easily displaced, the frequency was lowered by various amounts.

In some experiments we were able to observe that when air was introduced rapidly into the pleural cavity, or rapidly sucked out, the frequency of impulses was increased, or impulses were formed anew which normally occurred only at the changeover between the respiratory phases under conditions of forced respiration. A frequency increase of this kind is shown in Fig. 1, B. Evidently, in this case we are concerned with an increase in the activity of special receptors showing functional differences from those of the ordinary pulmonary stretch receptors.

Later experiments showed that when air was introduced into the pleural cavities there was also a change in the impulses which are grouped to coincide with the pulse, and which detailed investigations by many foreign

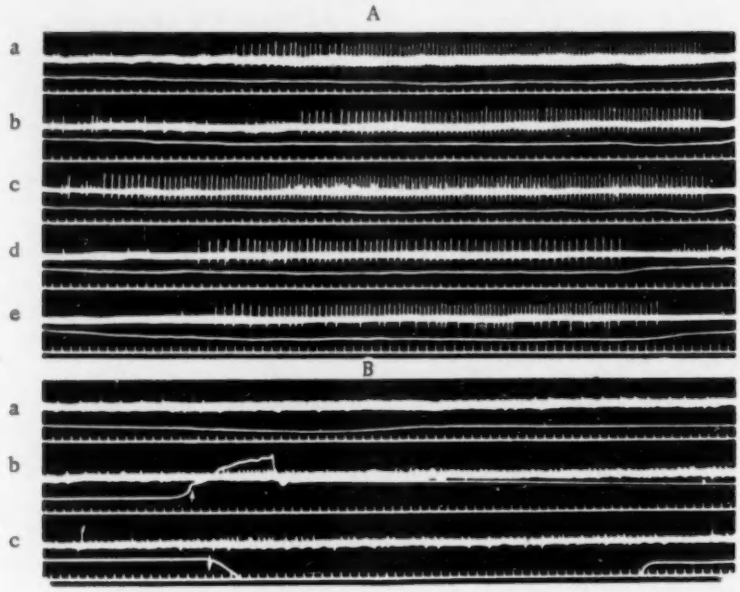


Fig. 1. Effect of establishing an artificial pneumothorax on the afferent impulses in a small bundle of fibers separated from the vagus in the neck on the side of the pneumothorax. Oscillograms A: a) impulses under normal breathing conditions; b) after introducing 10 ml per kg of air into the pleural cavity of the cat; d) the same 17 ml; c and e) after sucking our corresponding amounts of air; oscillograms B: a) impulses under normal breathing conditions; b) during rapid inflation of the pleural cavity with air; c) during the time when it is rapidly sucked out. Curves for each oscillogram (from top down): action potentials; variation of intrapleural pressure (a depression of the line corresponds to an inspiration); time marker (1/20 sec); arrow (↑) indicates the introduction of about 20 ml per kg weight of air into the pleural cavity, an arrow (↓) represents sucking the air out of the pleural cavity.

workers [15 and others] have shown to originate from cardiac receptors.

In some of the fibers, which appeared to be connected to receptors of the auricles (B-receptors), after air was introduced into the pleural cavities the impulse frequency was reduced. There was a reduction in the number of impulses occurring at each stroke, which might be due to a reduced venous return brought about by pneumothorax. The record of pressure in the femoral artery showed that there were no noticeable changes in arterial pressure (Fig. 2, A).

Other fibers, which probably mediate the activity of receptors responsive to pressure changes in the auricles (A-auricular receptors), the opposite result is obtained, and there is an increased frequency of impulses which is particularly well-shown during the inspirational phase (Fig. 2, B). The increase in the frequency of the pulses was greater, the greater the amount of air introduced into the pleural cavities.

To determine the effect of closed pneumothorax on the activity of the respiratory center we recorded action potentials at the central ends of fine bundles separated from the vagus in the neck region, or better still from its recurrent laryngeal branch. In the latter case, the afferent pathway from the thoracic cavity along the vagus nerve was preserved completely. In addition to fibers carrying efferent pulses at inspiration, it has also been shown [2, 7, 8, 12] that other vagal fibers carry pulses which effect expiration. Therefore by studying the activity of the fibers as described it was possible to obtain more information about the activity of the respiratory center than would have been possible by measuring the action potentials only in the phrenic nerve.

As can be seen from a typical oscillogram (Fig. 3, A), introduction of air into the pleural cavity causes first of all an increase in the frequency of impulses at inspiration. There may be either an increase in the frequency of impulses already present, or the development

of a new set of a different amplitude. The latter effect indicates the involvement of fresh neurones. After introducing air into the pleural cavity the duration of the first inspiratory discharges is frequently increased; the first inspiratory discharge after inflating the pleural cavity with air is particularly marked.

In addition to an increase of inspirational activity, the establishment of artificial pneumothorax also leads to an increase in neuronal discharge at expiration, which occurs after a short initial inhibition of this activity (Fig. 3, B). After sucking out the air from the pleural cavity, the activity of the neurones of the respiratory center both at inspiration and expiration is reduced, and returns to its original level.

We observed similar changes in the activity of the respiratory center in an experiment on a rabbit. It can be seen from the oscillogram of Fig 3, C, that introducing air into the pleural cavity causes an increased discharge at inspiration, indicating an increased activity of the corresponding neurones; impulses of a different amplitude occur during expiration, showing that neurones in the respiratory center concerned with expiration have become activated.

Thus an artificial pneumothorax reduces the frequency of the impulses originating in the pulmonary stretch receptors which form the typical volleys occurring at this phase. The effect is due to the lung being less dilated when a pneumothorax has been established than it is normally. D. A. Kocherga also observed a reduction in the frequency of afferent impulses in vagal fibers during the first few minutes after introducing air into the pleural cavity. On the other hand, V. M. Shirokaya described not a reduction but an increase in the volleys of impulses after the operation. By picking up the action potentials from bundles containing a large number of different nerve fibers, as V. M. Shirokaya did, it is not possible to determine which receptor endings are responsible for the increased electrical activity recorded. From the results which we have given here concerning the increase after pneumothorax of frequencies in certain fibers which terminate in the heart, it must be supposed that the increase of impulses observed by V. M. Shirokaya must be attributed to excitation of these cardiac receptors.

The activity of the respiratory center is increased by the pneumothorax which reduces the flow of impulses

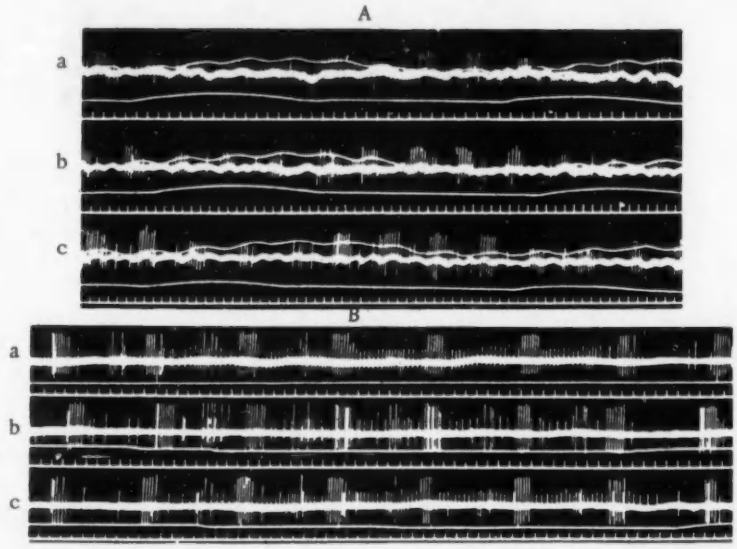


Fig. 2. Effect of establishing an artificial pneumothorax on the right side on the grouping according to the pulse rhythm of afferent impulses in the fibers of the right vagus of a cat. Oscillograms A and B: a) impulses under normal breathing conditions; b) after introducing 20 ml per kg of air into the pleural cavity; c) after sucking the air out of the pleural cavity. In oscillograms A, the upper line indicates the variations in general blood pressure (165-175 mm Hg); in oscillograms B, besides the impulses grouped at the pulse rhythm, there are also impulses from the pulmonary stretch receptors. Other indications as in Fig. 1.

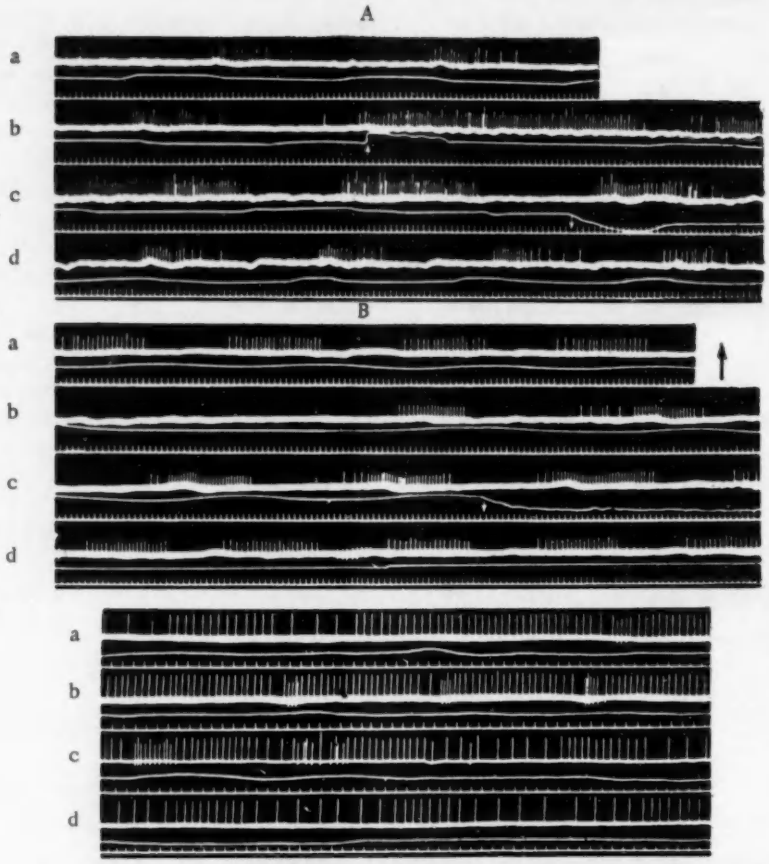


Fig. 3. Effect of establishing an artificial pneumothorax on efferent impulses in a bundle extracted from the receptor nerves of a cat (A and B) and a rabbit (C). Introduction of air into the pleural cavity is indicated by the arrows, as in Fig. 1, B. The air was forced in before part b was begun.

to it from the pulmonary stretch receptors. The effect is shown principally in an increased activity first of the neurones controlling inspiration, and then in those controlling expiration. Increased inspirational activity after introducing air into the pleural cavity is also observed when potentials are recorded from the phrenic nerve [5, 9, 14]. It is interesting that here also the neurones concerned with expiration also become more active. In considering the results from the point of view of the complex composition of the bulbar respiratory center [6] and the separate localization of inspiratory and expiratory neurones in the medulla [4, 11, 13, 16], it can be seen that there must be complex interrelationships between these two sets of neurones. The increase in activity of both sets following an artificial pneumothorax is evidently a compensatory reaction.

SUMMARY

Action potentials in the vagi of cats and rabbits were recorded to determine the effect of an artificial pneumothorax on pulmonary receptors and the respiratory center. It was found that there was a reduction in the impulses in the vagal fibers connected to pulmonary stretch receptors. The effect was to cause an increase in the activity of the respiratory center, which was shown at first by an increased number of efferent impulses at inspiration, and then an increase also in the impulses in the recurrent branch at expiration. It was also shown that the pneumothorax affected the cardiac receptors, and changed the frequency of the impulses which were grouped at the pulse rhythm.

LITERATURE CITED

1. M. I. Vinogradova, in: Annual Report of the Institute of Experimental Medicine, AMN SSSR [in Russian] (Leningrad, 1956) p. 113.
2. M. I. Vinogradova, in: Annual Report of the Institute of Experimental Medicine, AMN SSSR [in Russian] (Vil ' nyus, 1957) No. 4, p. 160.

3. M. I. Vinogradova, in: Problems of the Regulation of Respiration in Normal and Pathological Conditions [in Russian] (Moscow, 1959) p. 58.
4. I. A. Keder-Stepanova and G. A. Kurella, Fiziol. Zhur. 43, 1, 46 (1957).
5. D. A. Kocherga, in: Problems of the Regulation of Respiration in Normal and Pathological Conditions [in Russian] (Moscow, 1959) p. 137.
6. N. A. Mislavskii, The Respiratory Center [in Russian] (Kazan', 1885).
7. V. I. Filistovich, in: Annual Report of the Institute of Experimental Medicine, AMN SSSR [in Russian] (Leningrad, 1958) p. 100.
8. V. I. Filistovich, in: Problems of the Regulation of Respiration in Normal and Pathological Conditions [in Russian] (Moscow, 1959) p. 49.
9. V. M. Shirokaya, Reports of the Scientific Conference on Problems of N. E. Wedensky Parabiosis (Leningrad, 1957) p. 107.
10. V. M. Shirokaya, in: Problems of the Nervous Regulation of Function of the Animal and Human Organism under Normal and Pathological Conditions [in Russian] (Chita, 1958) No. 2, p. 71.
11. M. N. J. Dirken and S. Woldring, J. Neurophysiol. 14, 211 (1951).
12. J. H. Green and E. Neil, J. Physiol. 129, 134 (1955).
13. E. Haber, K. W. Kohn, and S. H. Hgai, et al., Am. J. Physiol. 190, 350 (1957).
14. M. G. Larrabel, G. C. Knowlton, Am. J. Physiol. 147, 90 (1946).
15. A. S. Paintal, J. Physiol. 120, 596 (1953).
16. R. F. Pitts, H. W. Magoun, and S. W. Ranson, Am. J. Physiol. 126, 673 (1939).

BLOOD COAGULATION IN C-AVITAMINOSIS

Communication III. The Effect of Vitamins C, P, B_{12}, K, and Folic Acid on the Thromboplastic Activity of the Blood of C-Avitaminotic Guinea Pigs

G. V. Andreenko and N. P. Sytina

Laboratory of Physiology and Biochemistry of Blood Coagulation (Dir. - Prof. B. A. Kudryashev) of the Soil Biology Department of the M. V. Lomonosov Moscow State University

(Presented by S. E. Severin, Active Member AMN SSSR)

Translated from the Byulleten' Éksperimental'noi Biology i Meditsiny, Vol. 50, No. 10, pp. 46-48, October, 1960

Original article submitted July 6, 1959.

In our previous reports [1, 2], we demonstrated that one reason for the development of hemorrhagic diathesis in C-avitaminosis is disturbance of blood thromboplastin formation. We observed this disturbance to be caused by a decrease in the thrombotropin (plasma component activating prothrombokinase) and prothrombokinase concentration in the blood platelets. A decreased concentration of prothrombin was also observed.

In his study of the effect of C-avitaminosis on the blood coagulation system, McCraw [7] also observed a decrease in the prothrombin concentration in the blood. McCraw believes the reason for the decrease of prothrombin to be a secondary K-avitaminosis which develops in an organism fed a diet devoid of vitamin C. The synthesis of prothrombin, like that of thrombotropin, is carried out by the parenchymal cells of the liver under the control of vitamin K. When vitamin K was administered to white rats suffering from K-avitaminosis and to normal rats, the concentration of prothrombin and thrombotropin in the blood was restored to the normal indices in the first case and rose above the normal level in the second case [4, 5].

With these facts in mind, we tested the effect of vitamin K on the thromboplastic activity and on the concentration of thrombogenic components in the blood of C-avitaminotic guinea pigs.

The prothrombokinase content of the blood can be increased by stimulating hematopoiesis and the formation of thrombocytes in the bone marrow. Vitamin B_{12} and folic acid exert such an effect. When administered to normal white rats, vitamin B_{12} sharply increases the thromboplastic activity of the blood – to 200% of the normal activity [3].

We administered vitamin B_{12} and folic acid to C-avitaminotic guinea pigs in an attempt to increase the thromboplastic activity of the blood by increasing the prothrombokinase content in the platelets.

We also studied the effect of vitamin P on the coagulating system of the blood, as some authors [6] believe scurvy to be polyavitaminosis of C and P rather than simply a monovitaminosis of C.

EXPERIMENTAL METHODS

The experiments were performed on guinea pigs weighing an average of 250-300 g. A condition of C-avitaminosis was induced in the animals by feeding them the diet devoid of vitamin C which we described in the earlier reports [1, 2]. Before and at fixed intervals after we started the experiment, blood samples were taken from the jugular veins of the experimental and control guinea pigs, and the thromboplastic activity and the thrombogenic components of the blood determined. The determination methods and the modifications we introduced in order to determine the percentile content of coagulation factors in the blood of the guinea pigs are described in the previous reports [1, 2].

Vitamin B_{12} was administered in a dose of 7.5γ per guinea pig; folic acid was given each animal in a dose of 2 mg; vitamin K was administered in the form of the hydrosoluble derivative Synkayvite in a dose of 50γ; vitamin P, in the form of citrin, and pure ascorbic acid were administered, respectively, in doses of 2.5 and 5 mg per animal. The vitamins were all injected intramuscularly in all the experiments except in one case, of which special mention will be made.

EXPERIMENTAL RESULTS

The experiments performed on 35 guinea pigs showed that the administration of vitamin K, vitamin B_{12} and folic acid did not prevent the development of C-avitaminosis and the associated disturbances of blood coagulation in the animals. The animals fed a C-avitaminotic diet and given a surplus of the above-mentioned vitamins lost weight and died on the 18th-20th day. The data in

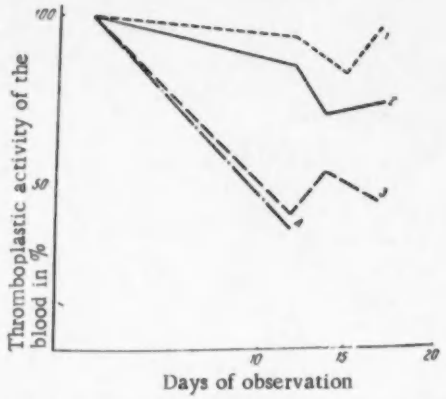


Fig. 1. Change in the thromboplastic activity of the blood of C-avitaminotic guinea pigs effected by the administration of vitamins C, K, B_{12} and folic acid. 1) Diet + 2.5 mg C; 2) diet + 7.5γ B_{12} + 2 mg folic acid; 3) diet + 50γ Synkayvite; 4) diet without additional vitamins.

the graph, however, indicate that vitamin B_{12} and folic acid do act to sustain the thromboplastic activity level of the blood. There was somewhat less change in the concentration of the thrombogenic components, prothrombin and thrombotropin, of the blood in this group than in the control. In one of the experiments, it was found that the efficiency of the vitamin C dose depends on the way in which it is administered to the organism. Although 2.5 mg, intramuscularly administered, is sufficient to effect normal development and weight gain in guinea pigs fed a C-avitaminotic diet, double this dose, i.e., 5 mg, administered per os is not sufficient to stabilize the weight. The administration per os of both vitamin C and vitamin P caused the animals to develop normally and to gain more weight than was observed with the intramuscular administration of ascorbic acid alone. The administration of vitamin P alone to the animals did not prevent the development of C-avitaminosis or the decrease in the thromboplastic activity of the blood.

It has already been demonstrated [1, 2] that the decrease in the thromboplastic activity of the blood which occurs in C-avitaminosis is due to the decrease in the concentration of thrombotropin and prothrombokinase in the blood. The results of the experiments with the administration of vitamins to animals fed a C-avitaminotic diet further demonstrate the determinant in the decrease of the blood's thromboplastic activity to be decrease in the prothrombokinase content. When hematopoiesis and the production of platelets – the source of prothrombokinase – were increased by the administration of vitamin B_{12} and folic acid, there was considerably less decrease in the thromboplastic activity of these scurvy guinea pigs than in that of the control.

The administration of vitamin K had no beneficial effect on either the thromboplastic activity of the blood or the thrombotropin level.

These data indicate that the decrease in the prothrombin and thrombotropin content of the blood of C-avitaminotic animals is evidently not due to a secondary K-avitaminosis, as McCraw proposed [7], but rather to a general decline of the synthetic processes which occurs in the absence of vitamin C.

SUMMARY

As shown by experiments conducted on guinea pigs fed a diet devoid of vitamin C, vitamin B_{12}, and folic acid administered in a daily dose of 7.5γ and 2 mg, respectively, diminished the extent of the blood thromboplastic activity reduction observed in these animals. With the administration of these vitamins, the concentration of prothrombin and thrombotropin decreased slightly less than in control experiments. Vitamin K rendered no favorable effect upon the concentration of thrombogenic components and thromboplastic activity of the blood. The efficacy of the vitamins stimulating hemopoiesis confirms the suggestion that the drop in blood thromboplastic activity is due to a reduced concentration of prothrombokinase in the platelets.

LITERATURE CITED

1. G. V. Andreenko and N. P. Sytina, Problemy Gematologii i Perelivaniya Krovi, 10, 26 (1959).
2. G. V. Andreenko and N. P. Sytina, Byull. Eksp. Biol. Med. 49, 3, 30 (1960).*
3. G. V. Andreenko and B. A. Kudryashov, Doklady Akad. Nauk SSSR 102, 4, 787 (1955).
4. B. A. Kudryashov, Doklady Akad. Nauk SSSR 60, 8, 1469 (1948).
5. P. D. Ulitina and B. A. Kudryashov, Doklady Akad. Nauk SSSR 63, 4, 465 (1948).
6. A. Benthsath, St. Rusznyak, and A. Szent-Gyorgyi, Nature 139, 326 (1937).
7. J. Y. McCraw., Rev. Cand. Biol. 14, 295 (1956).

* Original Russian pagination. See C. B. translation.

HEMATOPOIESIS AND VITAMIN B_{12} CONTENT IN DOGS AFTER GASTRECTOMY AND IN EXPERIMENTAL HEPATITIS

O. A. Korolenko

Department of Pathophysiology (Head-Prof. D. I. Gol'dberg) and the Department of Biochemistry (Head-Prof. L. D. Kashevnik) of the Tomsk Medical Institute
(Presented by V. N. Chernigovskii, Active Member of the AMN SSSR)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 48-53, October, 1960
Original article submitted August 14, 1959

Since the role played by disturbance of the formation of Castle's intrinsic factor in the pathogenesis of B_{12}-deficiency anemias has been explained [16, 18, 19, et al.), there has been greater interest in the experimental and clinical study of remote consequences of extensive resections of the stomach. Many descriptions of so-called agastric pernicious anemia in people who have undergone a total gastrectomy operation have appeared in recent years [2-5, 7, 8, 9, 23, 24, et al.]. Aside from Bence's attempts [12] to induce megaloblastic anemia in pigs after resection of the stomach, analogous experiments performed on other animals (dogs, monkeys) have shown the impossibility of developing a similar type of anemia experimentally [1, 6, 13, 14, 17, 20, et al.].

It was interesting in this connection to study the changes which occurred in hematopoiesis in an experiment on animals with a resected stomach and in which the vitamin B_{12}-depositing function of the liver was disturbed. Our experimental observations were performed on gastrectomized dogs in which we subsequently induced the development of toxic hepatitis.

EXPERIMENTAL METHODS AND RESULTS

Eleven of the twenty dogs used for the experiment survived. Six of the 11 surviving dogs had undergone total resection of the stomach; in the other five, the resection of the stomach was subtotal.

After the operation, we observed the development of a moderate hypochromic, normocytic anemia, which lasted 20-70 days and then usually subsided spontaneously. The hemoglobin decreased 10-23% – an average of 17.33%, – and the number of erythrocytes decreased 700,000-2,000,000, or an average of 1,350,000; no changes were observed in the number of reticulocytes and leukocytes. Only one dog (Bobka) died five months after total resection of the stomach, showing symptoms of pronounced hypochromic anemia (hemoglobin–32%, erythrocytes–4,060,000; see table). Examination of peripheral blood smears showed hypochromia and anisocytosis.

Examination of hematopoiesis in the bone marrow of the gastrectomized dogs disclosed the development in the animals of a hypogenerative reaction characterized by a decrease in the total number of erythroblasts and, to some extent, by disturbance of their maturation (relative increase of basophilic normoblasts and macroblasts and, sometimes, of proerythroblasts). The average erythrocyte diameter remained normal throughout the observation period in spite of a shift to the right (7.24-7.69 μ as against the initial 6.71-7.36 μ). According to the data of our department, the normal fluctuations for the average erythrocyte diameter in dogs are 6.2-7.7 ($M \pm 3\sigma$): $M = 6.95\ \mu$. We also observed this in our study of the vitamin B_{12} content in the blood serum and liver of the gastrectomized dogs (see table). The vitamin content was determined by V. N. Bukin's biological method. The content in the blood serum ranged between 252-381 $\mu\gamma$/ml (with background indices ranging from 319 to 532 $\mu\gamma$/ml). We determined the vitamin B_{12} content in the liver of the dogs at different times, depending on when the animals died (on the 74th day, after eight months and after one year), and established the following values: 251.26 mγ/g, 287.08 mγ/g and 259.06 mγ/g (the norm being 200-300 mγ/g). Using the dogs of this same group, we also studied certain of the liver functions. We found moderate disturbances of the functions studied – the proteinogenic function in particular. For example, the amount of total protein in the blood serum decreased 1-2% (from 7.2-6.89 to 5.9-5.25%); the mercuric chloride test decreased to 1.02-1.16 (the norm being 1.7-2), and the prothrombin content fluctuated between 83 and 89%. In a majority of analyses, Himans van den Bergh's test was negative, but the bilirubin content increased in a few cases to 1.8-2 mg%.

We induced the development of acute toxic hepatitis in some experiments in order to intensify the hematopoietic disturbances in the gastrectomized dogs. In this way, we proposed to effect disturbances of the vitamin B_{12} storage function of the liver and, on a background of gastrectomy, to be able to observe a megaloblastic shift of hematopoiesis.

The experiments were performed on three dogs (two with total, one with subtotal resection of the stomach). Hepatitis was induced by the intramuscular administration of a 1% yellow phosphorus solution in apricot oil,

injected in a dose of 0.01 g/kg. The injections were given twice a week; the course consisted of 4-5 injections.

The development of acute injury of the liver tissue was verified by an increase in the bilirubin content to 10.5-16.5 mg%, a decrease in the prothrombin content to 62.5-55% and a decrease in the mercuric chloride test to 0.8-1. The total protein content decreased to 4.2%.

Examination of the peripheral blood showed the development in these animals of hyperchromic macrocytic anemia, lasting 65-133 days (Fig. 1 a and b). The hemoglobin content fell 36-46% of the original figures; the number of erythrocytes decreased 2,000,000-2,790,000. There was a considerable increase in number of reticulocytes: this index had increased from 3-10‰ (before administration of the phosphorus) to 57-145.5‰ by the 31st-44th day of the experiment. As before, no changes in the number of leukocytes were observed. By the 65th-103rd day of the experiment, the average erythrocyte diameter had increased 0.7-0.92 μ, reaching 7.92-8.12μ. On erythrocytometric curves, the minimal diameter was 5-6μ, the maximal, 10-10.5μ. A few erythrocytes were as large as 11.5-12.5μ.

On supravitally stained peripheral blood smears, we also established that formations like Heinz bodies began to appear in the erythrocytes after the administration of the yellow phosphorus solution to the animals (Fig. 2 and 3). They did not appear until the 15th day of the experiment; they were first observed as single, very tiny formations, which then increased in size and number. The maximum number of erythrocytes containing these bodies was observed on the 18th-20th day of the experiment (984-871‰), after which the number of such erythrocytes began to decrease until, on the 45th-78th day of the experiment, the bodies were no longer apparent.

Therefore, this hitherto unrecorded appearance of pathological structures in the erythrocytes indicates that phosphorus is a hemolytic as well as a hepatic poison.

Bone marrow punctates taken from the dogs Dzhonka and Al'fa after the administration of the phosphorus showed an increased number of erythroblasts of every type, the immature forms in particular (proerythroblasts increased from 1.4 to 2.6%, basophilic erythroblasts increased from 2.75-8.4 to 16-19% and macroblasts increased from 4.6 to 32.6%). The leukoblast-erythroblast ratio decreased from 1.95-2.5 to 0.46-0.66. Erythropoiesis was observed to be inhibited in the dog Ryzhii (erythroblast content decreased from 41.6 to 11.4%), but there was a sharp increase in the number of reticuloendothelial and plasma cells (the former increased from 0.5-1 to 22%, the latter, from 1.8 to 6.8%.

The changes in the vitamin B_{12} content of the blood serum were very definite in character. There was a marked increase in the vitamin B_{12} content on the 15th-16th day of the experiment. In the dog Ryzhii, for example, two weeks after the administration of the phosphorus, the

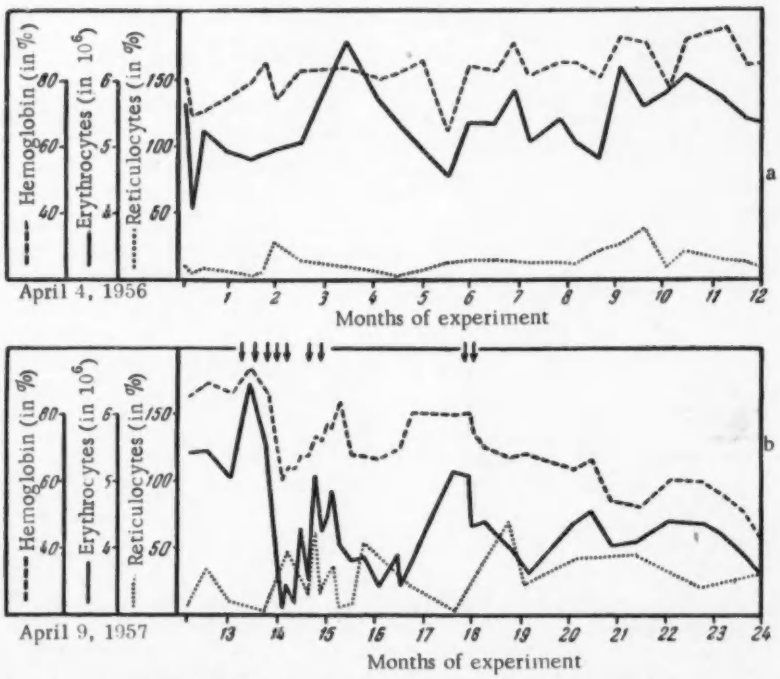


Fig. 1. Change in the blood indices of the dog Dzhonka (subtotal resection of the stomach and yellow phosphorus poisoning). a) During the 1st-12th months of the experiment; b) during the 13th-24th months of the experiment.

Maximal Changes in the Blood Indices and Vitamin B_{12} in Dogs Following Total or Subtotal Resection of the Stomach

Dog's name	Type of resection	Observation period	Hemoglobin content (in %)		Erythrocyte content		Average erythrocyte diameter (in μ)		Vitamin B_{12} content in blood serum	
			initial	minimum after resection	initial	minimum after resection	initial	minimum after resection	day of expt.	amount (in μγ/ml)
Dzhonka	Total	3 yr, 2 1/2 mos	81	68	5 600 000	4 120 000	7,09	7,33	396	305
Ryzhii	"	7 1/2 mos	106	75	6 000 000	3 820 000	7,36	7,69	90	243
Bobka	"	5 mos	80	32	6 220 000	4 060 000	7,26	7,57	—	—
Pudel'	"	50 days	97	80	5 890 000	4 210 000	—	—	67	293
Kukla	"	43 days	94	81	5 300 000	4 200 000	—	—	Bkgd.	387,5
Tarzan	"	28 days	88	72	5 750 000	5 380 000	—	—	—	—
Al'fa	Subtotal	1 yr, 4 1/2 mos (alive)	94	71	5 130 000	3 540 000	7,2	7,24	55	381
Pal'ma	"	1 yr, 7 1/2 mos	91	77	6 370 000	4 400 000	6,71	7,37	309	252
Pirat	"	7 1/2 mos	88	80	5 700 000	4 830 000	—	—	177	255
Naida	"	2 mos	90	79	5 180 000	4 380 000	—	—	—	—
Laima	"	34 days	10	90	5 810 000	5 050 000	—	—	Bkgd.	136

vitamin B_{12} content was twice the original (having increased from 243 to 580 μγ/ml) and by the 72nd day constituted 653.2 μγ/ml. In the dog Al'fa, the vitamin content had more than doubled by the 16th day, having increased from 381 to 776 μγ/ml, but the administration of phosphorus did not cause the same reaction in Dzhonka: in this dog, the vitamin B_{12} content did not increase until the 89th day of the experiment, and then only after an additional injection of the phosphorus was administered.

After involution of the symptoms of hepatitis and normalization of the liver functions, the vitamin B_{12} content in the blood of these dogs was back to normal. The normal hematologic indices and bone marrow picture were also restored. This restoration was not observed, however, in the dog Ryzhii, which died of poisoning on the 72nd day in a condition of serious hepatitis and anemia.

The increase in the vitamin B_{12} content of the blood serum effected by phosphorus poisoning was due to the fact that the storage function of the liver was disturbed during hepatitis. This conforms with clinical observations recording weakening of the storage function in patients with cirrhosis of the liver and infectious hepatitis [10, 11, 21, 22]. If the functional ability of the liver is subsequently restored, the possibility of vitamin B_{12} storage in it is also restored (normalization of the vitamin B_{12} content of the blood serum).

We also examined three control (non-gastrectomized) animals which had received yellow phosphorus in the same dose as the gastrectomized dogs. Macrocytic, hyperchromic anemia similar to that observed in the first group of dogs developed in these animals. The average erythrocyte diameter increased to 7.89-8.12 μ. The base of the Price-Jones curve ranged between 5.5-6.5μ and 10-10.5μ. The bone marrow punctates showed some decrease in the leukoblast-erythroblast ratio (from 1.09 to 0.94) due to a slight increase in the total number of erythroblasts: the polychromatophilic normoblasts increased from 8.3 to 10.3%, and the proerythroblasts increased from 0.6 to 1.6%. By the 15th day of the experiment, the vitamin B_{12} con-

Fig. 2. Blood smear from the dog Dzhonka after yellow phosphorus poisoning. Degenerate hemoglobin structures (supravitally stained with Nile blue sulfate).

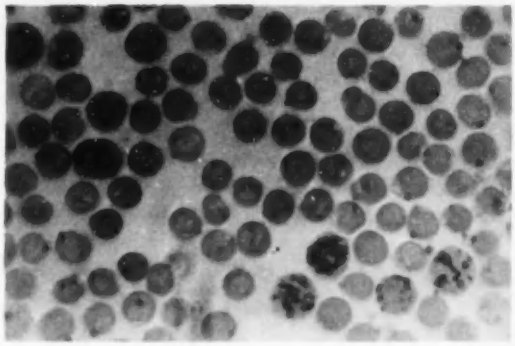


Fig. 3. Blood smear from the dog Al'fa after yellow phosphorus poisoning. Degenerate hemoglobin structures (supravitally stained with Nile blue sulfate).

tent in the blood serum of these dogs had increased from 532.2-516.5 to 1425-1212.5 $\mu\gamma$/ml, but was back to the original indices by the 51st day. In the liver of the dog Dina, which was killed on the 163rd day of the experiment, there was a decreased vitamin B_{12} content–94.83 mγ per 1 g fresh tissue.

From our observations, it is evident that phosphorus caused a more serious and protracted anemia in the animals which had undergone total gastrectomy. The administered dose of phosphorus proved lethal in the case of Ryzhii (total gastrectomy): the animal died, showing symptoms of acute macrocytic anemia, after receiving a dose easily tolerated by the non-gastrectomized dogs and the dog with subtotal gastric resection. Dzhonka (total gastrectomy) was killed by this same dose of the poison after its administration to her in the second experiment with phosphorus, performed almost a year after her first poisoning, with a picture of developed hepatitis and hyperchromic anemia.

Summing up the above, one can see that in spite of the absence of the stomach and the disturbance of the vitamin B_{12}-storing function of the liver, the macrocytic anemia and disturbance of the vitamin B_{12} content attending the experimental hepatitis were temporary changes, and no transition to the megaloblastic type of hematopoiesis was observed. This functional resistance of erythropoiesis is evidently due, to some extent, to the fact that in dogs Castle's intrinsic factor is formed in the small intestine as well as in the stomach.

SUMMARY

Experimental hepatitis was induced in gastrectomized dogs by the intramuscular injection of yellow phosphorus. Hepatitis development was associated with pronounced macrocytic anemia and disturbance of the B_{12} storing liver function. All these changes were only temporary. No transition to the megaloblastic type of hematopoiesis was observed. Analogous phenomena developed in dogs with experimental hepatitis and intact stomachs. Moreover, formations of the Heinz bodies type appeared in the erythrocytes as a result of the yellow phosphorus injection.

LITERATURE CITED

1. Ts. I. Abakeliya, Soobshcheniya AN Gruz. SSR 14, 10, 629 (1953).
2. G. A. Alekseev, Klin. Med. 11, 15 (1954).
3. G. A. Alekseev, Problemy Gematol. i Perelivaniya Krovi 5, 12 (1956).
4. A. A. Bagdasarov, P. M. Al'perin, M. Ya. Anshevits, et al., Problemy Gematol. i Perelivaniya Krovi 5, 3 (1956).
5. A. A. Bagdasarov, Vestnik AMN SSSR 3, 37 (1959).
6. Kh. Kh. Vlados, A. A. Bagdasarov, M. S. Dul'tsin, et al., in: Contemporary Problems of Hematology and Blood Transfusion [in Russian] (Moscow-Leningrad, 1936). Nos. 13-14, p. 41.
7. A. I. Gol'dberg, Transactions of the Fourth Pavlov Conference of the Tomsk Medical Institute [in Russian] (1954) p. 73.
8. A. I. Gol'dberg, Terap. Arkh. 1, 65 (1955).
9. A. I. Gol'dberg, Problemy Gematol. i Perelivaniya Krovi 2, 26 (1956).
10. N. T. Fokina, Problemy Gematol. i Perelivaniya Krovi 6, 33 (1956).
11. N. T. Fokina, Terap. Arkh. 5, 30 (1957).
12. J. Bence, Z. klin. Med. 130, 275 (1936).
13. R. A. Bussabarger, F. P. Cuthbert, and A. C. Ivy, J. Lab and Clin. Med. 24, 24 (1938).
14. R. A. Bussabarger, A. C. Ivy, and H. S. Wigodsky, et al., Ann. Intern. Med. 13, 1028 (1939).
15. W. B. Castle, Science 82, 159 (1935).
16. W. B. Castle, New England J. Med. 249, 603 (1953).
17. G. Fontès, J. Kunlin, and L. Thivolle, Sang 10, 433 (1936).
18. G. B. J. Glass, Therapie 10, 3, 425 (1955).
19. G. B. J. Glass, L. C. Lillick, and L. J. Boyd, Blood 9, 1127 (1954).
20. A. C. Ivy, O. Richter, and M. C. Kim, Am. J. Physiol. 101, 59 (1932).
21. M. Rachmilewitz, Y. Stein, and J. Aronovitch, et al., Arch. Intern. Med. 101, 1118 (1958).
22. E. E. Reimer, Wien. Z. inn. Med. 33, 303, (1952).
23. M. Tomoda, Chirurg 25, 49 (1954).

CHANGES IN THE BLOOD SYSTEM ASSOCIATED WITH DENERVATION OF THE DUODENUM

R. A. Durinyan

Laboratory of General Physiology of the Institute of Normal and Pathologic Physiology (Dir. – V. N. Chernigovskii, Active Member of the AMN SSSR) AMN SSSR, Moscow

(Presented by V. V. Parin, Active Member of the AMN SSSR)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 53-58, October, 1960

Original article submitted June 2, 1959

S. P. Botkin [1] was the first to recognize the possibility that the stomach, and, in particular, the duodenum, act reflexly on the composition of the blood. Presently there is no doubt about the close functional relationship between the organs of the digestive tract and the blood system. It has been shown, by a number of investigations [2, 3, 6, 8, 9, 11], that significant changes occur in the peripheral blood and bone marrow pictures as a result of either the removal of various portions of the digestive tract or the exposure of its mucosa to certain influences. These influencing factors often lead to the development of anemia. Reflex changes in the blood composition, in association with a diverse number of actions on the duodenal receptor apparatus, have been established as plausible [4, 5, 7, 8]. In our opinion, these observations testify to the importance, in the regulation of the blood system, of the efferent impulsation, and, in particular, the afferent impulsation, traveling from the receptor field of the duodenum.

In this investigation, we used the denervation method to exclude innervation and to exclude the afferent impulses in particular in order to study the effect of denervation of the duodenum on the blood system.

EXPERIMENTAL METHODS

The investigations were carried out as a chronic experiment on eight male cats; parellel investigations were conducted on the blood of six control cats. In each animal, the number of erythrocytes, leukocytes and reticulocytes, the percentage of hemoglobin, the osmotic resistance (fragility) of the erythrocytes, the volume of erythrocytes (hematocrit) and the average erythrocyte diameter were determined. The red marrow picture was studied in a few of the animals. The indices of the blood were studied for 3-4 weeks before the operation. Blood was taken from a small incision in the ear every other day until the third month after the start of the experiment, when it was taken every 3-4 days. Bone marrow was taken from the region of the upper third of the tibia while the animal was under general ether anesthesia. The investigations lasted 5-8 months. Denervation was performed as follows: all nerves terminating in the neu-

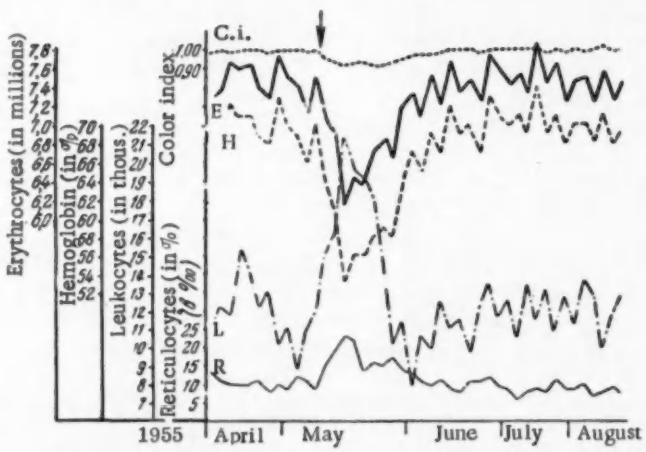


Fig. 1. Change in the peripheral blood after the control operation. E) Erythrocytes; H) hemoglobin; L) leukocytes; R) reticulocytes; C.i.) color index. Arrow shows the time of the operation.

rovascular bundle and going to the duodenum were cut; the adventitia was carefully removed from the vessels, which were then smeared with a 10% phenol solution. A layer of the serous membrane was annularly resected from the region where the pylorus meets the duodenum, and this section was also smeared with the 10% phenol solution. The control operation involved dissecting out the vessels and nerves of the same region.

EXPERIMENTAL RESULTS

The fluctuations in the blood indices of all the animals were slight before the operation (Fig. 1 and 2, to left of arrow).

The development of slight, short-lived anemia of the hyperchromic type, lasting 3-4 weeks, was observed after the control operation. All the blood indices returned to the original figures about the end of the first month and did not subsequently undergo any further change (see Fig. 1).

Substantial and considerable changes in the composition of the blood and bone marrow occurred after denervation of the duodenum. On the 2nd-3rd day after the operation, the erythrocytes increased 600,000-900,000 in number, but then decreased sharply on the 4th-8th day—by almost 2-3 million. Then, despite great fluctuations, the number of erythrocytes began to increase, in some cases even reaching the normal minimum; this period lasted 20-50 days, after which the number of erythrocytes began to decrease, variably but steadily, until at the end of the investigation their deficit constituted 2-2.5 million.

The changes in the hemoglobin content reflected those in the number of erythrocytes, but the decrease in the hemoglobin percent was less marked; the color index, therefore, began to increase gradually, and the initial hypochromic anemia was superceded by hyperchromic anemia (see Fig. 2). After the denervation, the number of reticulocytes increased from 9 to 30‰, and a shift of the reticulocytes to the left was observed. Towards the end of the second month, during the second decrease in the number of erythrocytes, the content of reticulocytes returned to the original level and subsequently remained fairly stable (see Fig. 2).

The osmotic resistance of the erythrocytes changed during the first period (minimal decreased 0.06%, maximal increased 0.04%), then returned to the original level (Fig. 3). The average erythrocyte diameter first decreased from 5.5 to 5μ, then gradually increased to 6.2μ (see Fig. 3). Characteristic changes occurred in the size and shape of the erythrocytes: during the initial period following denervation, the average volume and area of the erythrocytes decreased; the cells became thicker and microspherocytic in shape; then, during the period in which the development of hyperchromic anemia was observed, the average volume and area of the erythrocytes increased, the thickness diminished, and the cells took the form of macroplanocytes. The erythrocytometric curve also changed. The index of erythrocyte hemoglobin saturation decreased considerably (see Fig. 3).

The changes in the erythroid primordium of the bone marrow are described by the data presented in the Table, which show that erythropoiesis became inhibited after the denervation. This general retardation of erythropoiesis was attened by disturbance of the maturation process in stabe II of erythropoiesis. Therefore, denervation of the duodenum led to the development of anemia with a characteristic oscillation course.

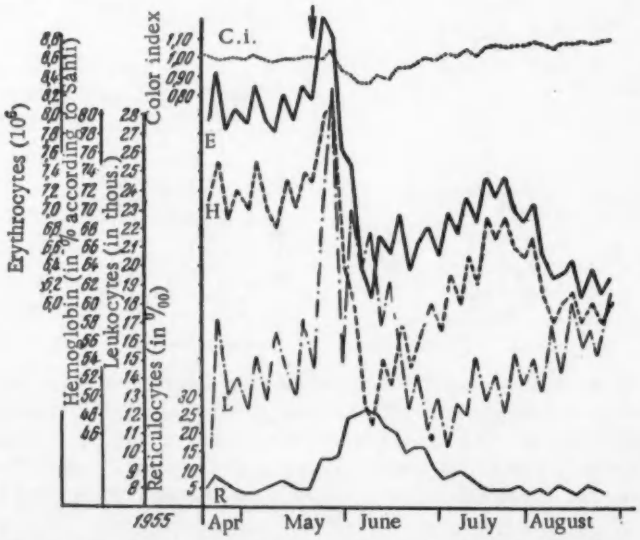


Fig. 2. Change in the peripheral blood after denervation of the duodenum. Symbols the same as in Fig. 1.

Change in Erythroid Primordium of Bone Marrow after Denervation of Duodenum (in % of 100 Bone Marrow Cells)

No. of experimental animal	Examination date*	Examination period	No. of dividing forms (mitoses)	Erythroblasts	Pronormoblasts	Normoblasts: basophilic	Normoblasts: polychromatophilic	Normoblasts: orthochromic	Reticulocytes	Number of erythrocytes in the peripheral blood
17	May 18	Bef. oper.	0.2	0.7	1.4	6.3	13.4	17.0	1.1	7 980 000
	Aug. 12	Aft. dener.	—	1.2	1.9	4.5	10.0	10.8	0.6	5 840 000
18	May 14	Bef. oper.	0.3	0.6	1.1	5.1	11.3	15.7	0.9	7 660 000
	Aug. 4	Aft. dener.	0.2	1.0	1.3	5.7	9.6	13.1	1.0	5 190 000
	Nov. 28		—	1.3	1.9	4.2	8.1	9.0	0.6	6 000 000
20	May 7	Bef. oper.	0.4	0.7	1.3	5.8	13.7	16.9	0.9	7 240 000
	Aug. 8	Aft. dener.	—	1.3	2.2	3.6	11.2	11.7	0.6	5 710 000

*All investigations conducted in 1955.

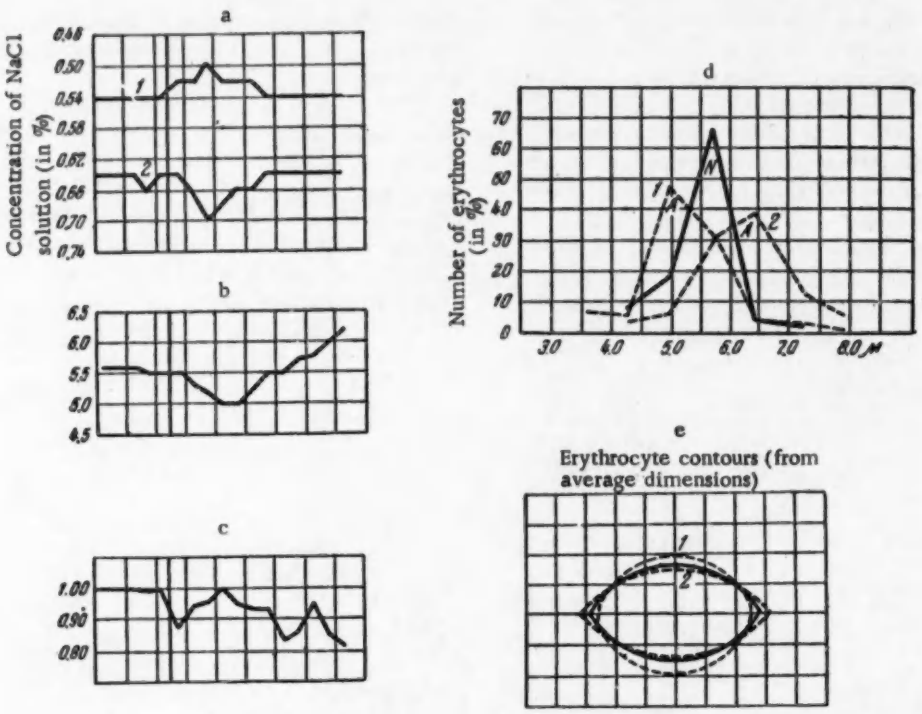


Fig. 3. Characteristic curves of the blood picture after denervation of the duodenum. a) Osmotic resistance of erythrocytes (1-maximal, 2-minimal); b) average erythrocyte diameter (in microns); c) index of erythrocyte hemoglobin saturation; d) erythrocytometric curve (N-before operation, A-during anemia); e) schematic shape of erythrocytes (according to average dimensions); 1-first wave of anemia, 2 -second wave of anemia.

During the first month following the operation, hypochromic, microcytic anemia of the regenerative type developed. Then hyperchromic, macrocytic anemia of the hyporegenerative type developed. The biphasic character of the anemia indicates diverse causes of its development. The first wave of anemia, hypochromic and microcytic in nature, was attended by marked reticulocytosis, a shift of the red blood to the left and great leukocytosis. Microspherocytosis and typical changes in the osmotic resistance were observed. All these changes developed under conditions of active erythropoiesis and were very typical of the hemolytic form of anemia. Similar, but less pronounced, changes were observed after the control operation. It would therefore appear that the initial mechanism of development of the anemia was the same with both denervation and the control operation and was caused by intensified hemolysis of the blood.

What are the reasons for intensified hemolysis? Every operative intervention is to some extent an injurious factor to begin with. The tissue in the vicinity of the denervation becomes a focus of intensified afferent impulsation, which produces a state of excitation in the central nervous system, and this, in turn, increases the activity of other systems, the blood system included (active erythropoiesis, reticulocytosis, leukocytosis). The intensification of the hemolytic processes is evidently stronger than that of the hemopoietic [5]. The spleen, which as the most labile organ in the blood system is considerably more sensitive to nervous influences, is irritated to a greater extent than the bone marrow tissue. It should be realized, however, that this mechanism cannot continue for long periods. The traumatic sequelae pass, and the original composition of the blood is restored. We believe this to be the mechanism of development of the anemia observed during the initial period after the operation.

Denervation stops the constant tonicizing impulsation from the intestinal receptors to the central nervous system and interrupts the efferent impulses. This diminishes the activity of the central nervous system apparatuses and weakens their inhibitory influence so that the blood system is, in a manner of speaking, "disinhibited" [8]. Under these conditions, the hemolytic function of the spleen is evidently intensified [5]. Of course, the disturbances induced in the central nervous system are not confined to the above.

The more or less identical character of the changes in the blood and bone marrow pictures after the control operation and during the initial period following denervation of the duodenum gives reason to believe that these changes were general in nature and cannot be considered to be specifically associated with denervation of the given organ. However, qualitatively new changes occurred soon after the first wave of anemia ended: a second, more stable and pronounced wave developed, this time of hyperchromic macrocytic anemia of the hyporegenerative type. There were no signs of hemolysis, and a low level of hematopoiesis was typical (see table). This could have been due to disturbance of the relevant efferent influences on the mucosa of the digestive tract from the central nervous system [8]. Disturbance of the relevant humoral mechanisms is also possible [10]. Therefore, during the second phase of development of anemia in the blood system, qualitatively new changes occurred which can be considered to be specific, associated with denervation of the duodenum.

SUMMARY

The effect of duodenal denervation upon the blood system was studied in conditions of chronic experiment (5-6 months) in 8 male cats. Six control male cats were investigated simultaneously. Two waves of anemia development were observed: the first—hypochromic, microcytic anemia of regenerative type; the second—hyperchromic, macrocytic anemia of hyporegenerative type. Some possible mechanisms of biphasic anemia development are discussed.

LITERATURE CITED

1. S. P. Botkin, Prof. Botkin's Clinical Lectures [in Russian] (St. Petersburg, 1885).
2. Kh. Kh. Vlados, A. A. Bagdasarov, M. S. Dul'tsin, et al., in: Contemporary Problems of Hematology and Blood Transfusion [in Russian] (Moscow-Leningrad, 1936) Nos. 13-14, p. 41.
3. D. L. Gol'dberg, Essays on Hematology [in Russian] (Tomsk, 1952).
4. A. Ya. Gubergrits, Autonomic Regulation of the White Blood [in Russian] (Kiev, 1941).
5. R. A. Durinyan, Experimental Analysis of Anemias Induced by Denervation of Certain Internal Organs. Author's Abstract of Candidate's Dissertation [in Russian] (Moscow, 1956).
6. E. L. Kan, Data Concerning the Study of the Influence of the Nervous System on the Composition of the Blood. Candidate's Dissertation [in Russian] (Leningrad, 1953).
7. E. M. Semenovskaya, et al., in: Contemporary Problems of Hematology and Blood Transfusion [in Russian] (Moscow, 1952) No. 31, p. 87.
8. V. N. Chernigovskii and A. Ya. Yaroshevskii, Questions Concerning Nervous Regulation of the Blood System [in Russian] (Moscow, 1953).
9. H. Braunsteiner, E. Gisinger, and F. Pakesch, Acta haemat., 10, 46 (1953).
10. E. Komiya, Die Zentralnervöse Regulation des Blutbildes (Stuttgart, 1956).
11. M. Paulson and F. Harvey, J. AMA 156, 1556 (1954).

THE INVOLUTION OF EXPERIMENTAL CHOLESTEROL ATHEROSCLEROSIS IN DOGS

A. N. Fedoseev

Department of Clinical and Experimental Physiology (Head-V. V. Parin, Active Member of the AMN SSSR) of the Central Institute of Graduate Medical Studies of the Soviet Union Ministry of Public Health (Dir. - M. D. Kovrigina)

(Presented by V. V. Parin, Active Member of the AMN SSSR)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 58-61, October, 1960
Original article submitted April 11, 1960

In investigations carried out on ten male dogs, three to four years of age, we established the dynamics of change in certain hemodynamic indices during the development of experimental cholesterol atherosclerosis and compared them with biochemical, pathologicoanatomic and histological data [3, 4].

This article presents the results obtained from investigations carried out on dogs after the animals had been fed cholesterol and 6-methylthiouracil for a 120-day period. The purpose of these experiments was to demonstrate the dynamics of the involution of functional and morphological changes caused by experimental athero sclerosis.

To this end, we continued to examine the dogs by every method at our disposal after the termination of the cholesterol and 6-methylthiouracil feeding period.

A month after we discontinued feeding the above substances to the animals, the general condition of the dogs began to improve gradually. The animals became more alert to their surroundings and considerably more active, and the hypothyroid symptoms decreased quite sharply. Dyspnea developed less frequently.

Cholesteremia decreased considerably, but continued to exceed the original levels, being on the average, double the latter. The maximal arterial pressure continued, as in the primary experiment, to vary from measurement to measurement. Some improvement was observed in the indices of the ballistocardiograms and electrocardiograms. An increase was observed on the ballistocardiograms in the amplitude of the waves, which was decreased during the feeding period, while the electrocardiograms showed an increase in voltage of the waves in all leads. The expansion rate of the pulse wave in the dogs did not change after we stepped feeding them cholesterol and 6-methylthiouracil.

From the works of N. N. Anichkov's school [1] and those of other researchers [5], it is known that the vascular changes which develop in experimental cholesterol atherosclerosis of rabbits and dogs undergo involution and, therefore, that atherosclerosis is a reversible process. On the basis of this very important concept, complex investigation of two dogs of the same series was continued for six months; the administration to them of cholesterol and 6-methylthiouracil was then discontinued.

We continued for six months to record the electrocardiogram (in three standard leads) and the ballistocardiogram, to determine the spread velocity of the pulse wave and to measure the maximal arterial pressure before and after the infliction of a physical load on these dogs (No. 9 and No. 10). The physical load inflicted consisted of a five minute run on a treadmill, the belt of which moved at a rate of 6.5 km/hr. We continued regular determinations of the cholesterol content of the animals' blood (using Grigo's method).

Definite signs of the involution of atherosclerosis were observed during our six-month observation of dogs No. 9 and No. 10. The dogs became lively and cheerful; they reacted to changes in their environment and showed signs of playfulness. The weight of the dogs (Table 1), which had decreased during the feeding period, began to increase slowly, but six months after the end of the feeding period was still 1.5-2 kg less than it had been before cholesterol feeding.

The cholesterol content of the blood returned to the original levels two months after the end of the feeding period and then remained stable from this time on (Table 2). The maintenance of an increased level of cholesteremia for a considerable period following the end of the cholesterol and 6-methylthiouracil feeding period is interesting.

The persistence of hypercholesteremia for two months after the administration of cholesterol to the experimental animals was discontinued indicated, in our opinion, that this condition was not caused simply by the organ-

TABLE 1. Weight of Dogs (in kilograms) during Involution of Atherosclerosis

Animal No.	Weight at end of feeding period	Change in weight after feeding period					
		after 1 month	after 2 mos	after 3 mos	after 4 mos	after 5 mos	after 6 mos
9	17.0	17.4	18.0	18.2	19.1	19.4	19.5
10	17.5	17.0	17.7	18.0	19.7	19.2	19.4

TABLE 2. Cholesterol Content (in mg%) in the Blood of Dogs during Involution of Atherosclerosis

Animal No.	End of feeding period	Months following					
		1	2	3	4	5	6
9	504	216	180	132	144	144	144
10	240	180	168	144	132	156	144

ism's increased intake of cholesterol. The organism's reaction to the cholesterol overload was evidently associated with a more profound disturbance of the animal's metabolic processes, primarily, the processes of lipoid-cholesterol metabolism. During the initial period following the end of 6-methylthiouracil administration, however, the hormonogenic function of the thyriod gland remained depressed, so that the cholesterol content of the blood could have continued to be increased.

The maximal arterial pressure continued to fluctuate within the physiologic range from experiment to experiment as it did during the preliminary investigation and during the period the animals were fed cholesterol and 6-methylthiouracil [3]. No stable increase in the blood pressure was observed, although it usually increased somewhat after the treadmill run.

After the cholesterol and 6-methylthiouracil feeding of the animals was stopped, the dogs' ballistocardiograms, which had shown considerable changes during the feeding period [4], approximated the original indices more rapidly and more completely than did the electrocardiograms.

Six months after the animals were last fed the experimental substances, their ballistocardiograms were still somewhat flattened (Fig. 1). The electrocardiograms of these dogs continued, as during the feeding period , to differ considerably from the initial recordings (Fig. 2).

The electrocardiogram continued to show the inversion of the T wave in the second and third leads, flattening of the P wave and biphasic state of these waves, and these were particularly marked after the physical load. According to the ballistocardiographic data, therefore, normalization of the contractile function of the myocardium occurred more fully and rapidly than was evident from the electrocardiograms, in which the changes remained considerable. These facts are very important to the prognosis of the clinical course of atherosclerosis. During the six-month period of involution of the atherosclerotic changes, the spread velocity of the pulse wave continued to be, as before, 7.05-10.8 m/sec [4].

Dogs No. 9 and No. 10 were killed at the conclusion of the experiment.

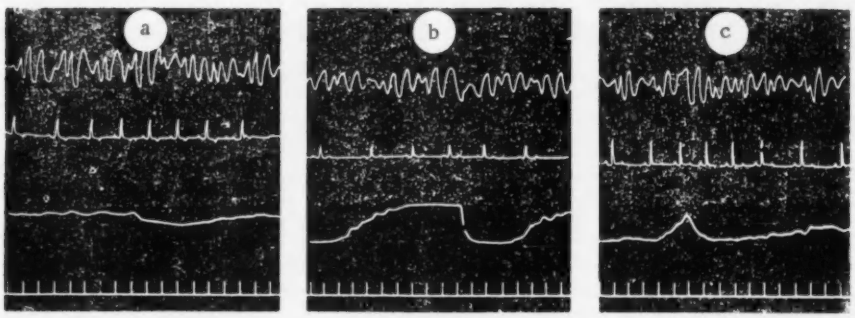


Fig. 1. Change in direct ballistocardiogram of heart rate of dog No. 9 under the different experimental conditions. a) Before feeding; b) during feeding; c) six months after the administration of cholesterol and 6-methylthiouracil was stopped. Curves (from top to bottom) represent: ballistocardiogram; lead II of ECG; respiration; time in $^1/_4$ - second marks.

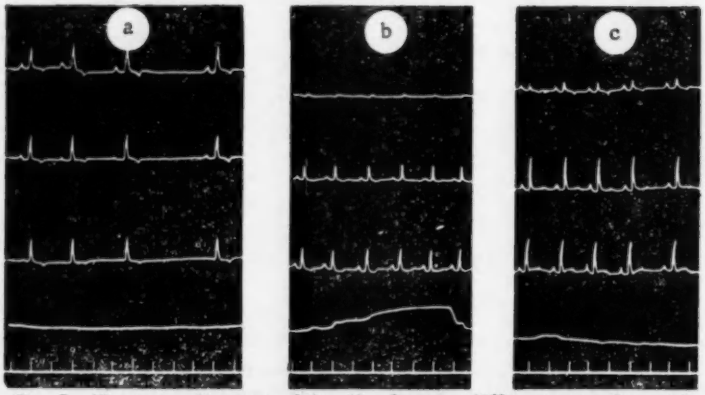


Fig. 2. Electrocardiogram of dog No. 9 under different experimental conditions. a) Before feeding; b) during feeding; c) six months after the administration of cholestrol and 6-methylthiouracil was stopped. Curves (from top to bottom) represent: ballistocardiogram; lead II of ECG; respiration; time in $^1/_4$-second marks.

Pathologico-anatomic and histological investigations of the cardiovascular system and internal organs of these dogs, performed six months after the end of the cholesterol and 6-methylthiouracil feeding period, disclosed that all the changes found earlier in the other animals of this series were considerably less pronounced in these two dogs [3]. The small quantity of atherosclerotic plaques found on the internal surface of the aorta, carotid and other arteries contained very little fat and were observed to be in a state of cicatrization. The fat deposits had disappeared almost completely from the internal organs. The microscopic picture of the thyroid gland, which (in the other dogs of the series and the dogs of the control group which had received 1.5 g of 6-methylthiouracil apiece) had a characteristic structure during the administration of 6-methylthiouracil [3], was fully normalized. Slight sclerotic changes were observed in the liver; still less expressed sclerotic changes were found in the heart muscle. The latter explain the presence of stable changes on the electrocardiograms of these dogs six months after the last feeding of cholesterol and 6-methylthiouracil to the animals.

Macroscopic and microscopic morphological investigations, performed a month after the last cholesterol and 6-methylthiouracil feeding, showed a picture of extensive atherosclerosis in all the animals of this series [3].

SUMMARY

Over a six-month period following the end of the cholesterol and 6-methylthiouracil feeding period, the ballistocardiographic changes in dogs went back more rapidly and more completely to the initial level than the electrocardiographic changes.

Pathological and histological investigations of the cardiovascular system and internal organs of these animals showed involution of atherosclerotic lesions.

High blood cholesterol 2 months after the administration of cholesterol was stopped points to important metabolic damage.

LITERATURE CITED

1. T. A. Sinitsyna, Abstracts of the Proceedings at the Session on the Problem: Atherosclerosis and Myocardial Infarction [in Russian] (Moscow, 1958) p. 31.
2. A. N. Fedoseev, Byull. Éksp. Biol. Med. 46, 12, 101 (1958).*
3. A. N. Fedoseev, Byull. Éksp. Biol. Med. 50, No. 11 (1960).†
4. A. N. Fedoseev, Byull. Éksp. Biol. Med. 50, 5, 41 (1960).*
5. M. Bevans, et al., Arch. Path. 51, 278 (1951).

* Original Russian pagination. See C. B. translation.

† See C. B. translation.

ON THE QUESTION OF CHANGES IN THE ACID-BASE BALANCE AND THE REDOX PROCESSES IN PERITONITIS ASSOCIATED WITH BLOOD LOSS

E. M. Neiko and L. Ya. Fishchenko

Department of Pathophysiology (Head-Prof. N. V. Kolpikov)
of the Stanislavskii Medical Institute (Dir. - Docent G. A. Babenko)
(Presented by Active Member AMN SSSR V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 10, pp. 62-63, October, 1960
Original article submitted August 14, 1959

The purpose of this work was to trace the changes in the acid-base balance and the blood catalase indices in peritonitis associated with blood loss. The presence of this type of data affords the opportunity of explaining the pathogenesis of these two pathological processes.

The work was carried out on 35 rabbits. The entire experimental stock was divided into three series. In the first series (10 rabbits) we studied the indices associated with peritonitis. In the second series (20 rabbits) we studied the same indices in association with peritonitis plus the loss of 15% of the blood volume. The third series (5 rabbits) served as the control to the blood loss factor. The animals were maintained for three days under identical regimes and diets, and their temperature, leukocyte count, hemoglobin in percent, erythrocyte count, blood catalase and alkaline reserve were all determined prior to the beginning of the experiment. After the investigations were performed in the rabbits of the first and second series the operative field was prepared and a laparotomy executed under local anaesthesia (10-15 ml of 0.5% novocaine subcutaneously). A perforation was made in the stomach, using a rod 0.3 cm in diameter that was first heated "red hot." With this, the contents of the stomach poured out into the peritoneal cavity. One billion microbial bodies from a one-day-old culture of a virulent strain of Staphylococcus aureus, seeded from the purulent exudate of patients, were introduced into the lumen of the stomach via the perforation. The same microbial emulsion, intro-

The Change in a Number of Blood Indices (in Average Numbers) Observed in Rabbits with Peritonitis, Peritonitis in Association with a Blood Loss of 15%, and a Blood Loss without Peritonitis

Series	Experimental conditions	Body temperature	Leukocytosis	Hemoglobin (in Sahli units)	Erythrocytes	Catalase index	Alkaline reserve (in vol % CO_2)
First	Original state	38,2°	9470	74	4 090 000	11,66	34,3
	Peritonitis	39,4° (+1,2°)	24 650 (+15,18)	72 (—2)	3 695 000 (—395 000)	9,86 (—1,80)	30,0 (—4,3)
Second	Original state	38,8°	9850	73	4 598 000	10,84	30,0
	Peritonitis and a 15% blood loss	39,6° (+0,8°)	17 360 (+7,51)	52 (—21)	2 910 000 (—1 688 000)	7,66 (—3,18)	19,4 (—10,6)
Third	Original state	39,4°	9060	76	4 414 000	10,48	41,7
	15% blood loss without peritonitis	39,2° (—0,2°)	7630 (—1,43)	71 (—5)	3 916 000 (—498 000)	8,74 (—1,74)	35,8 (—5,9)

duced subcutaneously into a rabbit, caused necrosis and pustular inflammation of the cellular tissue; and, when injected intravenously, death. The wound in the peritoneal cavity was sutured tightly. In addition, in the animals of the second and third series the needle of a syringe was inserted into the cavity of the heart and blood was withdrawn up to an amount representing 15% of the total blood volume. The original amount of blood in this case was taken to be $^1/_{13}$th of the animal's weight. The results of the blood loss were evaluated by the changes in the quantity of hemoglobin and the number of erythrocytes in the blood. The temperature and blood leukocytosis served as indices of the development of the inflammation. Catalase was determined by the method of Bakh and Zubkovaya; alkaline reserve was measured by the method of van Slyke. The animals, prepared in this manner, were again subjected to the determinations after 24 hours. The results obtained from these studies are presented in the table.

EXPERIMENTAL RESULTS

As can be seen from the table, the peritonitis caused by this method was accompanied by an elevation in the temperature and the leukocyte count, a lowering of the erythrocyte count, the blood catalase index and the blood alkaline reserve. In the case of peritonitis in association with a loss of 15% of the total blood volume the elevation in the temperature and the leukocyte count was less marked, while the remaining indices (erythrocyte count, percent hemoglobin, blood catalase index and alkaline reserve) underwent more appreciable changes. In the animals of the third series, which were subjected to only the blood loss, the temperature even fell slightly; the rest of the indices changed even less significantly than in the second series. Inflammation and blood loss by themselves caused a lowering of the blood catalase. The combination of inflammation (peritonitis) with blood loss was accompanied by a lowering of the blood catalase that was almost twice as strong as that caused by either peritonitis or blood loss alone. We noted similar changes in connection with the blood alkaline reserve.

On the basis of the data we obtained it may be concluded that peritonitis in association with blood loss and blood loss alone diminish the body temperature the leukocyte count, the percent hemoglobin, and the erythrocyte count.

Lower values are observed for the catalase index and the alkaline reserve in rabbits with peritonitis plus blood loss.

The reactivity of the animals with peritonitis complicated by blood loss differs from the reactivity of animals in which the blood loss did not take place.

SUMMARY

The loss of 15% of the blood volume accompanying infectious peritonitis weakened the leukocytic and the temperature reaction in rabbits and decreased the phagocytic activity of leukocytes. In rabbits with blood loss and peritonitis there were less leukocytes in the peritoneal exudate than in those without any such loss; there was a rise of the acidity and protein level in the exudate. Arterial pressure dropped (both spontaneously and after intravenous administration of the exudate) – more so in peritonitis complicated by blood loss than in an uncomplicated one.

THE CHARACTERISTICS OF THE NUCLEAR NUCLEOPROTEINS OF SOME EXPERIMENTAL TUMORS AND OF CHICK EMBRYOS

I. B. Zbarskii and L. P. Ermolaeva

Group on the Biochemistry of Cell Structure (Head-Prof. I. B. Zbarskii), A. N. Severtsev Institute of Animal Morphology (Dir.-Corresponding Member AN SSSR Prof. G. K. Khrushchov) Academy of Sciences of the USSR, Moscow
(Presented by Active Member AMN SSSR S. E. Severin)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 64-67, October, 1960
Original article submitted January 21, 1960

According to investigations carried out in our laboratory, the cell nuclei of tumor tissues actually differ in protein and nuclein composition from the nuclei of normal cells [3-7, 12, 13]. These differences are not found in nuclei isolated from the cells of embryonic tissues [4, 6, 7, 12]. A number of authors have mentioned the difference in deoxyribonucleoprotein complexes (DNP) of tumors [11, 12], which are evidently due to the relatively high content of nonhistone, tryptophan-containing proteins.

The characteristics of the tumor nuclear nucleoproteins have been demonstrated by immunological methods [10, 11]. Also, in tumors some differences are observed in the properties of histones [15].

These investigations indicate that the nuclear nucleo proteins of tumors differ from the normal, which may be very significant for the nature of tumor growth. It should be remarked, however, that the above results were obtained by different methods which were not always reliable. The composition of the nuclear nucleoproteins depends to a great extent on the method of their isolation [17], and the preparation of DNP in the native state is of no little difficulty [2, 14].

The investigations of Vendrely and co-workers show the homogeneity in DNP composition of different tissues, but a great difference is found in this depending on the presence of histones or protamines in the composition of DNP [19, 22].

Considering a number of deficiencies in method in the earlier experiments, chiefly connected with the method of obtaining the isolated nuclei [21], which could result in a change in the DNP composition, we studied the nuclear nucleotides of some tissues, isolating the nuclei and DNP preparations exceedingly carefully in an attempt to preserve the original composition. In the study mentioned [9] we succeeded in showing that DNP samples from different organs of the rat and chick scarcely differ from each other in many respects, but have a different composition from the DNP of sturgeon sperm, which contains protamines. The composition of the DNP which we obtained differed, however, from that in the investigations of the above mentioned authors [19, 20].

In the present work we have studied the DNP obtained from isolated cell nuclei of some primary tumors and from chick embyros.

METHOD

We studied DNP obtained from isolated cell nuclei of whole chick embryos on the seventh and eleventh day of incubation, the liver of 19-day chick embryos, the cells of Ehrlich ascitic tumor of mice (on the seventh day after transfer) and of Walker rat carcinosarcoma 256. The cell nuclei were separated by the two-step sucrose-glycerophosphate method [1]. The nuclei from the tumors contained a pyroninophilic residue, evidently of cytoplasmic origin. Therefore the nuclei from the Ehrlich ascitic tumor cells were partially separated by the method of osmotic shock [18].

According to the method which we have described [8, 9] the globulin fraction is fully removed from the isolated nuclei, and then the DNP is extracted and precipitated by dilution with water [9].

Analysis of the resulting DNP preparations was carried out in the same way as in the work mentioned [9].

RESULTS

The composition of the DNP is given in Table 1 in percentage of the DNP preparation under conditions of 16% nitrogen content. The nitrogen content was determined on the air-dried preparation.

The content of diamino acids in the DNP preparation is given in Table 2.

The results which we obtained showed that DNP from chick embryos in practice does not differ from the earlier-studied DNP from 11-day chick embryos, liver of 19-day embryos and adult chickens, and DNP of rat organs [9]. Like the DNP mentioned, the nitrogen/phosphorus ratio is here 4.0-4.8, the DNA content does not exceed 40%, the

TABLE 1. Composition of DNP Separated from Isolated Nuclei (in Percent of DNP Containing 16% Nitrogen)

Source of preparation	Nitrogen	Phosphorus	N/P	DNA	Arginine	DNA/Arginine	No. moles arginine per atom DNA phosphorus
7 day whole chick embryo	14.0	3.1	4.5	39.4	7.8	5.05	0.38
11 day whole chick embryo	13.6	3.4	4.0	40.2	7.4	5.45	0.35
Liver of 19 day chick embryo	14.0	2.9	4.8	38.5	7.3	5.26	0.36
Walker carcinoma 256	12.0	3.2	3.8	46.3	7.2	6.44	0.30
Erlich ascitic tumor:							
sucrose-glycerophosphate method	11.5	4.5	2.6	46.5	6.3	7.39	0.26
osmotic shock method	14.4	3.6	4.0	41.1	7.0	5.88	0.33
	11.3	4.5	2.5	52.0	9.8	5.30	0.36
	11.0	4.2	2.6	51.7	10.2	5.06	0.38

TABLE 2. Content of Diamino Acids in DNP Obtained from Isolated Nuclei

Source of preparation	No. g amino acid per 100 g DNP contg. 16% N			No. molecules amino acid per atom DNA phosphorus			
	Arginine	Lysine	Histidine	Arginine	Lysine	Histidine	Total diamino acids
7 day whole chick embryo	7.8	8.0	1.0	0.378	0.462	0.055	0.895
11 day whole chick embryo	7.4	7.3	2.0	0.352	0.414	0.107	0.873
Liver of 19 day chick embryo	7.3	8.3	1.3	0.362	0.490	0.072	0.924
Walker carcinoma 256	7.2	8.2	1.3	0.297	0.403	0.060	0.760
Ehrlich ascitic tumor:							
sucrose-glycerophosphate method	6.3	7.6	1.2	0.258	0.372	0.054	0.684
osmotic shock method	7.0	8.5	1.2	0.325	0.470	0.062	0.857
	9.8	11.7	1.4	0.360	0.513	0.058	0.931
	10.2	13.1	1.0	0.376	0.576	0.041	0.993

DNA/ arginine ratio is 5.0-5.5, and there is about 0.35 mole arginine per atom of DNA phosphorus.

DNP obtained from tumor nuclei was less constant in composition. The first three preparations, the DNP obtained from Walker carcinoma , the Ehrlich ascite tumor cells obtained using sucrose - glycerophosphate and that from the ascitic tumor obtained by the osmotic shock method,were close in composition to "normal" and "embryonal" DNP. They differed in having a higher DNA content and somewhat less arginine. Correspondingly, the nitrogen/phosphorus ratio was somewhat lower, and the DNA/arginine somewhat higher. Per atom of phosphorus,there was a somewhat smaller number of moles of arginine. These differences were small and the small number of DNP preparations and the considerable differences between them did not create assurance of the significance of these differences. The differences between the preparations might depend on the fact that they were obtained by one method from different tumors and by different methods from one tumor. Nevertheless, this result shows the necessity for a more careful comparison of the composition of tumor and normal DNP.

On the other hand, the last two preparations, obtained from the nuclei of Ehrlich ascitic tumor cells isolated by the method of osmotic shock, actually differed in high content of DNA, arginine, and lysine, low nitrogen/ phosphorus ratio and large number of total diamino acids per atom of phosphorus of DNA. It should be noted that in obtaining these two preparations the DNP solution had a relatively low viscosity, and when diluted with water precipitated only a small amount of DNP. This fact leads us to assume that the last two preparations have undergone a partial splitting, probably because of breakdown of the nonhistone protein. The assumption certainly requires testing, but there are a number of indications in the literature of considerable changes in DNP composi-

tion with modification of the method of its separation [17] and also with its reprecipitation by dilution with water [12].

Thus, in the present study on the materials of DNP of chicken embryos we have confirmed the conclusions of our previous work [9] both as to the homogeneity of DNP of different tissues and the fact that original DNP has per atom of DNA phosphorus 0.8-0.9 mole of diamino acids. This value agrees with the data of Davison and Butler [16] and considerably exceeds the value of 0.65 mole of diamino acids given by Vandrely [20]. Though the results of our work for tumor DNP do not show a difference in its composition, yet they indicate with great probability the possibility of such differences. Our results also confirm the difficulty of separating DNP in the original state and the existence of differences in DNP composition depending on the method of obtaining the cell nuclei. This fact is evidently the reason for the variations in literature data on the composition of DNP and the peculiarities of tumor DNP.

The explanation of the individuality of composition of tumor DNP can be very important for the pathogenesis of malignant tumors. The evidence for such differences requires surmounting a number of methodological difficulties.

SUMMARY

Deoxyribonucleprotein (DNP) obtained from isolated cellular nuclei of chick embryos as well as the DNP of the liver of adult hens and rats contained 40% of DNA (the ratio DNA/arginine being 5.0-5.5 with 0.8-0.9 moles of diamino acids per atom of DNA phosphorus). Tumor DNP's differed in having a higher content of DNA and a lower percentage of arginine. In two samples with low viscosity and probably degraded, the DNA and diamino acid content was particularly high, which stresses the dependence of the composition of the DNP on the conditions under which it is obtained.

LITERATURE CITED

1. G. P. Georgiev, L. P. Ermolaeva, and I. B. Zbarskii, Biokhimiya 25, 2, 318 (1960).
2. A. L. Dounce, in: Contemporary Problems of Cytology [in Russian] (Moscow, 1955) p. 51.
3. S. S. Debov, Biokhimiya 16, 4, 314 (1951).
4. S. S. Debov, in: Problems of Medical Chemistry [in Russian] (Moscow, 1953) Vol. 6, p. 117.
5. I. B. Zbarskii, in: Progress of Biological Chemistry [in Russian] (Moscow, 1950) Vol. 1, p. 91.
6. I. B. Zbarskii, in: Problems of Clinical and Experimental Oncology [in Russian] (Stalingrad, 1957) Vol. 2, p. 96.
7. I. B. Zbarskii in: Malignant Neoplasms [in Russian] (Moscow, 1959) p. 54.
8. I. B. Zbarskii, and G. P. Georgiev, Biokhimiya 24, 2, 192 (1959).*
9. I. B. Zbarskii, and L. P. Ermolaeva, Biokhimiya 25, 1, 114 (1960).*
10. L. A. Zil'ber, V. B. Freiman, I. B. Zbarskii, et al., Doklady Akad. Nauk SSSR 65, 1, 97 (1949).
11. S. B. Manoilov, in: The Problem of the Nature of Nucleoproteins of Normal Tissues and Malignant Tumors, Doctoral Dissertation [in Russian] (Leningrad, 1951).
12. K. A. Perevoshchikova, in: Problems of Medical Chemistry [in Russian] (Moscow, 1953) Vol. 6, p. 125.
13. S. M. Saidov. Byull. Éksp. Biol. Med 45, 1, 45 (1958).*
14. J. A. V. Butler, and P. F. Davison in: Advances in Enzymology (New York, 1957) Vol. 18, p. 161.
15. H. J. Cruft, C. M. Mauritzen, and E. Stedman, Philos. Tr. Roy. Soc. London. Ser. B., 241, 678, 93 (1957).
16. P. F. Davison, and J. A. V. Butler, Biochem. et biophys. acta 21, 568 (1956).
17. Y. Khouvine, and F. Baron, Grégoire, et al., Bull. Soc. chim. biol 36, 31 (1954).
18. R. M. S. Smellie, H. M. Keir, and E. D. Gray, et al., Biochem. J. 72, 17,(1959).
19. C. Vendrely, A. Knobloch, and R. Vendrely, Biochim. et. biophys. acta. 19, 472 (1956).
20. C. Vendrely, A. Knobloch, and R. Vendrely, Compt. rend. Acad. Sci, 246 3128 (1958).
21. R. Vendrely, and C. Vendrely, Experientia 4, 434, (1948).
22. R. and C. Vendrely, Compt. rend. Acad. Sci. 235, 395, (1952).

* Original Russian pagination. See C. B. translation.

THE PROBLEM OF THE EFFECT OF PARASYMPATHETIC DENERVATION ON THE PROTEIN METABOLISM OF THE SUBMAXILLARY SALIVARY GLANDS OF THE CAT

G. V. Chernysheva

Laboratory of Biochemistry (Head – Prof. V. M. Rubel'), Institute of Normal and Pathological Physiology (Director – Active Member AMN SSSR V. N. Chernigovskii) AMN SSSR, Moscow

(Presented by Active Member AMN SSSR V. N. Chernigovskii)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 67-73, October, 1960

Original article submitted October 14, 1959

The problem of the effect of various sections of the nervous system on the secretory function of the salivary glands has been studied in considerable detail. Less studied has been the effect of the nervous system on the metabolic processes which occur in the gland tissue [2, 4, 5, 19, 23-27]. This is especially true of protein metabolism, which plays an important part in the secretory processes of the gland [7, 8, 10, 21]. In addition, the study of nitrogen metabolism of the salivary gland and its regulation by the nervous system is of great value for the understanding of the mechanism of secretion.

The problem of the present work is the investigation of protein metabolism in the submaxillary salivary gland and its comparison with the secretory function of this gland after destruction of its connection with the central nervous system by section of the chorda tympani.

EXPERIMENTAL METHOD

The experiments were carried out on the submaxillary salivary glands of cats. We ran several series of experiments with 20-30 animals in each. Parasympathetic denervation (one or both sides) was carried out in the same way as in a number of other studies by sectioning the chorda tympani where it is given off from the lingual nerve and goes to the submaxillary gland [13, 22, 24, 26], thus breaking basically the parasympathetic and partially the afferent connection with the center. The study was carried out on the tissues of the salivary gland and on the saliva. Nitrogen content of the saliva was studied in cats with a submaxillary gland fistula. Exposure of the duct was carried out by the method of A. M. Ugolev [9]. Tissue proteins were studied in the gland removed rapidly after decantation of the fluid. The gland was weighed, frozen with dry ice (to reduce the action of tissue enzymes) and ground to a fine powder. All further treatment of the tissue was carried out in the cold. A sample of tissue was ground in a mortar, homogenized in a glass homogenizer in a 0.2% solution of KH_2PO_4 with pH 5.33 (2.5 ml of solution per g of wet tissue). Then the homogenate was centrifuged for 10 minutes at 5000 rpm. In the resulting water-salt extract we determined the total nitrogen (by Kjeldahl), mucin nitrogen (by the method of Anrep [10] and Kazakova [6]), and the amount of nitrogen in other proteins, not precipitated with mucin. For precipitation we used 7% CCl_3COOH.

To characterize the protein fractions we determined in them after hydrolysis with 2 N HCl at 100° for 4 hours the content of amino sugars by the method of Elson and Morgan [12] and the pentosuria method [3], and also the amount of reducing substances by the Hagedorn method.

RESULTS

The study of the tissues and saliva of the submaxillary gland after unilateral denervation was carried out on the 8th, 14th, and 21st days and after bilateral denervation, only on the 21st day after sectioning the chorda tympani. We chose the latter period because at this time there occur in the gland the greatest changes in its secretion [2, 11, 18, 20].

Sectioning the chorda tympani caused a short reflex formation of saliva on eating meat. In our experiments, as in those of Emmelin and Muren [13-16] the "paralytic" secretion of saliva was absent.

In the table we give the average data for each series of experiments. As controls for the denervated gland we used the glands of intact cats.

The content of nitrogenous substances was calculated per g of dry tissue and per weight of the whole gland. As the table shows, on the 8th to 9th day after unilateral sectioning of the chorda tympani the denervated gland does not show any marked differences in weight, amount of solid residue, or nitrogen content of its tissues. Later on after the denervation there was progressive fall in weight of the gland. The amount of solid residue decreased on the 14-15th day and remained small on the 21st day after denervation. In the tissues of the denervated gland the content of total nitrogen and mucin nitrogen, calculated per g of dry weight, was higher than in the tissues of the glands from intact cats (50.1 mg of mucin nitrogen on the 14th day after denervation, 45.6 mg on the 21st day, and 38.6 mg in the intact cats). The content of protein which did not precipitate with the mucin decreased on the 14-15th day after denervation. On the 21st day it was within normal limits.

Change in Chemical Composition of Tissues of Submaxillary Glands of Cats under the Influence of Denervation

Experimental conditions			Weight of one gland, mg	Solid residue, %	Total nitrogen, mg		Mucin nitrogen, mg		Protein nitrogen without mucin, mg		Ratio of protein N without mucin to mucin nitrogen, %	Amino sugars		Reducing substances		No. of animals		
					per g dry tissue	in one gland	per g dry tissue	in one gland	per g dry tissue	in one gland		mucin (mg per g dry tissue)	protein without mucin (mg/g dry tis)	mucin (mg per g dry tissue)	protein without mucin (mg/g dry tis)	mucin (mg per g dry tissue)	protein without mucin (mg/g dry tis)	No. of animals
In resting state			980	23.4	52.4	12.25	38.6	7.7	3.6	0.7	10.0	37.2	—	84.7	2.4	0.5	0.02	43
After injection of pilocarpine			887	20.0	51.0	9.1	27.8	5.5	9.8	2.1	26.8	21.5	0.6	78.0	5.4	0.4	0.03	34
After unilateral denervation	on 8–9th day	contralateral gland	991	24.1	61.5	14.6	48.4	11.5	2.9	0.7	6.2	26.0	0.2	—	—	3,4	—	15
		denervated gland	914	23.3	52.0	11.0	39.0	8.2	3.0	0.6	6.8	20.0	1.0	—	—	1.7	0,32	15
	on 14–15th. day	contralateral gland	1 017	23.4	59.6	14.2	46.45	11.2	3.7	0.8	11.2	124.4	0	—	—	—	—	11
		denervated gland	711,7	20.7	62.8	9.25	50.15	7.35	2.0	0.3	5.5	87.1	—	—	—	0.6	—	11
	on 21st day	contralateral gland	916	22.5	66.1	13.3	44.9	9.8	4.8	1.3	13.7	—	—	78.7	4.3	—	—	37
		denervated gland	666	21.1	61.5	9.3	45.6	6.6	4.1	0.6	10.5	—	—	90,7	3.9	0.6	0.22	37

Change in Chemical Composition of Tissues of Submaxillary Glands of Cats under the Influence of Denervation (continued)

Experimental conditions			Weight of one gland, mg	Solid residue, %	Total nitrogen, mg		Mucin nitrogen, mg		Protein nitrogen without mucin, mg		Ratio of protein N without mucin to mucin nitrogen, %	Amino sugars		Reducing substances		Pentoses		No. of animals
					per g dry tissue	in one gland	per g dry tissue	in one gland	per g dry tissue	in one gland		mucin (mg per g dry tissue)	protein without mucin (mg/g dry tis)	mucin (mg per g dry tissue)	protein without mucin (mg/g dry tis)	mucin (mg per g dry tissue)	protein without mucin (mg/g dry tis)	
With injection of pilocarpine in unilateral denervation	after 1 hour action	contralateral gland	876	22.5	50.0	9.65	30.8	5.1	17.1	1.9	45.9	15.0	1.6	—	—	0.5	0.36	14
		denervated gland	764	18.35	48.3	6.4	32.4	4.3	10.8	1.1	50.1	8.5	—	—	—	0.6	0.8	14
	after 2 hour action	contralateral gland	1 071	21.7	62.5	13.9	43.35	9.95	8.7	2.1	23.6	16.4	0.2	52.0	6.5	—	—	16
		denervated gland	913	16.0	70.0	10.9	42.2	7.2	13.1	2.0	30.1	35.4	—	61.6	5.6	—	—	16
After bilateral denervation		denervated gland	726	23.6	64.8	10.6	52.5	9.2	2.9	0.4	4.4	12.8	—	100	2.4	—	—	23
With injection of pilocarpine in bilateral denervation	after 2 hour action	denervated gland	749	18.0	50.5	7.0	29.3	4.2	6.7	1.0	23.4	39.5	17.7	—	—	2.0	11.6	16

We must note that although the contralateral gland differed little in weight and quantity of solid residue from the gland of the intact cats, the content of mucin nitrogen in the extracts of these tissues was considerably higher than in the tissues from the glands of intact cats.

The increased content of nitrogenous substances which we found in tissues of denervated glands on the 21st day after unilateral sectioning of the nerve was still more marked in this period with bilateral denervation. The content of mucin nitrogen in the tissues of these glands rose to 52.5 mg (in unilateral denervation, 45.8 mg; in intact cats, 38.6 mg).

Thus, from all these experiments with unilateral and bilateral sectioning of the chorda tympani we can demonstrate the accumulation of mucin nitrogen in the tissues of the denervated glands. Evidently this is due to the absence of consumption of protein, since after denervation there is neither reflex nor "paralytic" secretion.

In order to follow the manner in which denervation acts on the function of the gland, we studied the secretion of this gland after injection of pilocarpine (0.75 mg per kg weight of animal) before and after section of the chorda tympani.

The study was carried out on 12 cats with fistulas of the submaxillary glands. Saliva was collected with a small funnel attached by Mendeleev cement at the point of emergence of the duct. Every 30 min we observed the amount of saliva and determined its content of total nitrogen and mucin nitrogen. In these experiments the change in secretory activity of the denervated gland was most sharply shown with bilateral section of the chorda tympani.

In the figure we give different typical experiments. The action of pilocarpine on glands with severed chorda tympani decreased the latent period to 3-5 min compared to a latent period of 14-15 min in these glands before denervation. The latent period of the contralateral glands as a result of unilateral denervation did not change and was the same as in glands which retained their innervation (14-15 min).

When pilocarpine acted on the denervated glands it was found, just as in the experiments of Pierce and Gregersen [22], that there was a shift in maximum formation of saliva: before sectioning the secretory nerve, the greatest quantity of saliva in response to a pilocarpine injection occurred in the second half hour of secretion; and, after sectioning, in the first half hour. The total amount of saliva formed after injection of pilocarpine after $1\frac{1}{2}$ hr secretion was the lower, the greater the period of time which passed after denervation (see figure, black columns). This was also observed by Vulpian [28] and by Langley [20]. However, the amount of total nitrogen (see figure) and mucin nitrogen isolated from saliva in the time of secretion after injection of pilocarpine was considerably greater in the entire period of denervation (8th, 14th, 21st day) than before denervation of these glands under the same action of pilocarpine.

The increase in content of mucin nitrogen in unilateral and bilateral denervation probably is connected with an increase in sympathetic influence which, as is known, occurs in parasympathetic denervation of the glands [2, 15, 17].

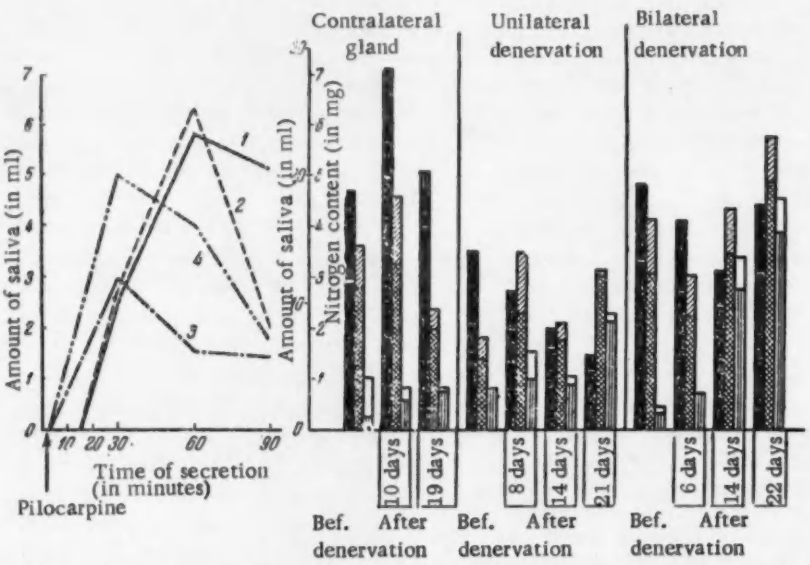
Thus, the study of the secretory function of the denervated glands has shown that the glands after sectioning of the chorda tympani in response to the action of pilocarpine form less saliva, but saliva with a greater content of nitrogenous substances than before the operation.

The high content of protein in the saliva formed after injection of pilocarpine into denervated glands leads to an understanding of the amount in the tissues of these glands. The more mucin that is excreted with the saliva, the less of it remains in the gland tissue. This is particularly clear after bilateral denervation of the gland (the content of mucin nitrogen in the tissues falls from 52.5 mg to 29.3 mg) and after an hour's action of pilocarpine on the denervated gland of an animal with unilateral sectioning of the nerve (from 45.6 to 32.4 mg). After two hours action, the content of mucin nitrogen in the tissues of denervated glands with unilateral denervation decreases only slightly (from 45.6 to 42.2 mg). This is apparently connected with the fact that the excretion of mucin with the saliva occurs especially in the first hour of secretion (see figure).

During the second hour of secretion, with injection of pilocarpine there is formed a liquid low in the nitrogenous substances of saliva (see figure, upper part of the columns). These facts allow us to suggest that the content of protein in the tissues after two hours' secretion on pilocarpine, strongly reflects the restoration of the mucin lost in the first hour of secretion.

Under the influence of pilocarpine on the denervated glands, in their tissues, just as in the tissues which have retained their innervation, there is a strong increase in the amount of protein nitrogen which does not precipitate with mucin (see table). We showed previously [1] that the appearance of this protein is connected with strengthened secretory activity of the gland. This protein gives reactions characteristic for glucoproteins, to which group submaxillary mucin belongs, but in its ability to be precipitated and in its content of amino sugars, pentoses, and total reducing substances, it differs from mucin (see table). The increased content of this protein can be compared with the data of histological study of denervated glands after the action of pilocarpine [16]. The authors have shown an increase of serous cells and a raised content in them of secretory granules.

Thus, all of the experiments run with the action of pilocarpine on denervated glands show that denervation does not decrease the synthesis of proteins and the excretion of the salivary glands, but, as compared with intact glands, changes the intensity of metabolic processes



Change in secretion and amount of nitrogenous substances of saliva after injection of pilocarpine before and after denervation of the submaxillary gland. 1) Secretion of saliva (in ml) before denervation; 2) secretion of saliva by the contralateral gland; 3) secretion of saliva after unilateral denervation; 4) secretion of saliva after bilateral denervation: Black columns—total amount of saliva formed after two-hour secretion with pilocarpine. Slanted line columns—content of total nitrogen (in mg) in this saliva. White columns—content of mucin nitrogen. Lower part of all columns (lower cross-hatched part)—amount of saliva, total nitrogen, and mucin nitrogen formed in first-hour secretion with pilocarpine.

and their relation to each other in response to the same stimulation.

SUMMARY

The author studied the influence of unilateral and bilateral section of the chorda tympani on the protein metabolism and the secretory function of the submaxillary salivary glands in cats. The reflex and the "paralytic" secretion in the denervated glands was absent. The nitrogen content of mucin in the tissues of these glands increased.

Changes in protein metabolism and in the secretory activity of the glands were more marked with bilateral denervation. Denervation did not stop secretion and synthesis of protein. Administration of pilocarpine caused typical changes in the salivary secretion and in the content of nitrogenous substances in the saliva and in the tissue of the salivary glands: 1) the latent period decreased; 2) the maximum quantity of the secreted saliva was changed; 3) after denervation the total amount of saliva secreted in 2 hours decreased (the content of the nitrogenous substances, however, was much higher than before denervation); 4) after pilocarpine the nitrogen content of mucin in the tissues of the denervated glands decreased and the content of protein which was not precipitated with mucin and which is typical of an actively secreting gland increased.

Thus, the parasympathetic denervation alters protein metabolism and the secretory function of the submaxillary salivary glands in cats.

LITERATURE CITED

1. M. P. Apanasyuk, M. S. Morozova, V. M. Rubel', et al., Abstracts and Reports, Ninth Congress of All-Union Society of Physiologists, Biochemists, and Pharmacologists [in Russian] (Moscow-Minsk, 1959) Vol. 2, p. 22.
2. B. P. Babkin, Secretory Mechanism of the Digestive Glands (New York, 1944).
3. A. N. Belozerskii and N. L. Proskuryakov, Practical Handbook of Plant Biochemistry [in Russian] (Moscow, 1951).
4. M. A. Guberniev, L. G. Kovyrev, and M. D. Ushakova, Doklady Akad. Nauk SSSR 95, No. 6 1251 (1954).
5. K. S. Zamychkina, Arkh. Biol. Nauk. 34, 1-3, 105 (1934).
6. M. E. Kazakova, Biokhimiya 17, 2, 195 (1952).
7. L. M. Lindenbaum, Trudy Ukrainsk. Psikhonevrologicheskogo Instituta, (Kharkhov, 1927) No. 4, 133
8. I. P. Pavlov, in: Complete Collected Works [in Russian] (Moscow, Leningrad, 1946) Vol. 2, p. 276.
9. A. M. Ugolev, Byull. Éksp. Biol. Med 40, 8, 76 (1955)
10. G. V. Anrep, J. Physiol. 54, 319 (1921).
11. J. R. Bradford, J. Physiol., 8, 86, (1887).

12. L. A. Elson, and W. T. J. Morgan, Biochem. J. 27, 1824 (1933).
13. N. Emmelin and A. Murèn, Acta physiol. scandinav. 24, 103 (1951).
14. N. M. Emmelin and A. Muren, Acta physiol. scandinav. 20, 13 (1950).
15. N. Emmelin, Physiol. Rev. 32, 21 (1952).
16. N. Emmelin, D. Jacobsohn, and A. Muren, Acta physiol. scandinav. 24, 128 (1951).
17. A. J. Fleming and F. C. Macintosh, Quart. J. Exper. Physiol. 25, 207 (1935).
18. R. Heidenhain, and B. Luchsinger, in: L. Hermann, Handbuch der Physiologie (Leipzig, 1879) Vol. 5, 87.
19. L. E. Hokin and M. R. Hokin, Canad. J. Biochem. and physiol. 34, 349 (1956).
20. J. N. Langley, J. Physiol. 7, 371 (1885).
21. G. O. Langstroth, et al., Proc. Roy. Soc. London 125, 335 (1938).
22. F. R. Pierce and M. L. Gregersen, Am. J. Physiol. 120, 246 (1937).
23. L. H. Schneyer and C. A. Schneyer, Am. J. Physiol. 187, 403 (1956).
24. L. H. Schneyer and C. A. Schneyer, Am. J. Physiol. 189, 129. (1957).
25. B. C. R. Strömblad, Acta physiol. scandinav. 36, 158 (1956).
26. B. C. R. Strömblad, Acta physiol. scandinav. 36, 158 (1955).
27. B. C. R. Strömblad, Acta physiol. scandinav. 145, 551 (1959).
28. A. Vulpian, cited by N. Emmelin (1952).

THE EFFECT OF CARBON DIOXIDE GAS ON THE CONTENT OF AMMONIA, GLUTAMINE, AND UREA IN THE BLOOD OF ANIMALS AFTER INJECTION OF SOLUTIONS OF AMMONIUM CHLORIDE

N. B. Kozlov

Department of Biochemistry (Head-Prof. V. I. Panisyak),
Smolensk Medical Institute
(Presented by Active Member AMN SSSR V. V. Parin)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 10, pp. 73-78, October, 1960
Original article submitted March 25, 1960

It was shown in an earlier study that in ammonia intoxication which developed in warming animals, carbon dioxide gas has a good therapeutic effect. In the present work we give results of experiments on the study of the effect of carbon dioxide gas on the concentration of ammonia, glutamine, and urea in the blood in animals with ammonia intoxication developed after injection of a solution of ammonium chloride.

EXPERIMENTAL METHOD

The experiments were carried out on 29 rabbits. The solution of ammonium chloride was injected subcutaneously in doses of 0.1, 0.25, 0.4, and 0.5 g per kg. In order to determine the effect of carbon dioxide gas on the blood content of the nitrogenous components studied, the rabbits were placed for one hour in a special glass chamber with capacity of 500 liters. The concentration of carbon dioxide gas in the chamber was 7-8%; the oxygen concentration was 18.5-19.5%.

Blood was taken for study from the marginal vein of the ear. In the blood we determined: ammonia according to Conway, glutamine by the method of Harris, urea by the urease method, and pH by the electrometric method.

RESULTS

In the first series of experiments we studied the effect of carbon dioxide gas on the content of ammonia, glutamine and urea in the blood of intact animals. The blood in the experimental animals in this series was taken before placing them in the chamber with the carbon dioxide gas and immediately after taking them out of it. The results are given in Table 1.

As Table 1 shows, an hour's exposure of rabbits in an atmosphere with 7-8% carbon dioxide gas does not cause a change in the concentration of blood ammonia, glutamine, or urea.

TABLE 1. Effect of Carbon Dioxide Gas on Content of Ammonia, Glutamine, and Urea in Blood of Intact Animals (Duration of Keeping in the Chamber with the Carbon Dioxide Gas, One Hour)

Rabbit No.	Ammonia nitrogen (in mg%)		Amide N of glutamine (in mg%)		Urea nitrogen (in mg%)	
	Before keeping in chamber	After keeping in chamber	Before keeping in chamber	After keeping in chamber	Before keeping in chamber	After keeping in chamber
1	0.20	0.19	0.75	0.76	15.0	15.0
2	0.23	0.24	0.52	0.51	17.0	20.0
3	0.17	0.16	0.57	0.61	17.0	17.0
4	0.06	0.08	—	—	22.0	23.0
5	0.15	0.18	0.90	0.87	15.0	14.0
Average	0.16	0.17	0.69	0.69	17.2	17.8

In the next series of experiments the rabbits were injected subcutaneously with ammonium chloride in the doses mentioned earlier. Each rabbit was studied twice. In one case, after injection of ammonium chloride, the rabbit remained under ordinary atmospheric conditions (such experiments we called the controls), and in another, after injection of the same dose of ammonium chloride, the rabbit was at once placed for one hour in the chamber with the carbon dioxide gas. The blood in the animals of this series was taken before injection of ammonium chloride, and one hour and two hours after injection of the solution. In the experiments using carbon dioxide, the second sample was taken at the moment of removing the animal from the gas chamber. The results of the experiments are given in Tables 2 and 3.

TABLE 2. Effect of Carbon Dioxide Gas on Concentration in the Blood of Ammonia, pH of the Blood, and on the State of the Animal after Injection of Solutions of Ammonium Chloride

Animal No.	Wt, kg	Dose ammonium chloride, g/kg	Control experiments						Experiments using carbon dioxide gas					
			ammonia nitrogen, mg %			blood pH		State of animal and result	Ammonia nitrogen (in mg %)			Blood pH		State of animal and result
			before injection	aft. injection after 1 hr	aft. injection after 2 hr	before injection	after 1 hr		before injection	aft. injection after 1 hr	aft. injection after 2 hr	before injection	after 1 hr	
6	2.6	0.1	0.20	0.43	0.10	—	—	Good	0.20	0.33	0.22	—	—	Good
7	2.7	0.1	0.27	0.37	0.24	—	—	»	0.26	0.30	0.23	—	—	»
8	2.3	0.1	0.14	0.50	0.26	—	—	»	0.10	0.13	0.06	—	—	»
9	2.6	0.25	0.21	1.88	0.81	—	—	Convulsions	0.28	1.10	0.45	—	—	»
10	2.7	0.25	0.36	1.34	0.73	—	—	Ataxia	0.38	0.93	0.49	—	—	»
11	2.6	0.25	0.28	1.83	0.90	—	—	Convulsions	0.32	1.51	0.73	—	—	Ataxia
12	2.1	0.25	0.13	0.88	0.18	—	—	Good	0.08	0.36	0.09	—	—	Good
13	2.2	0.25	0.08	0.96	0.34	—	—	Ataxia	—	—	—	—	—	—
14	2.0	0.25	0.12	0.91	0.18	—	—	»	0.08	0.38	0.18	—	—	Good
15	2.1	0.25	0.09	1.70	0.23	7.56	7.32	Convulsions	0.15	1.36	—	7.66	7.41	Ataxia
16	2.7	0.25	0.12	1.55	—	7.53	7.01	»	0.20	0.85	—	7.53	7.40	Good
17	2.3	0.25	0.19	0.60	—	7.49	7.45	Good	0.08	0.79	—	7.49	7.26	Satisfactory
18	2.1	0.25	0.18	1.04	—	7.53	7.27	Ataxia	0.19	0.69	—	7.44	7.32	Good
19	2.0	0.4	—	—	—	—	—	Convulsion, death	—	—	—	—	—	—
20	1.8	0.4	0.17	2.55	2.89	—	—	Convulsion	—	—	—	—	—	—
21	1.9	0.4	—	—	—	—	—	—	0.13	1.42	0.51	—	—	Satisfactory
22	3.1	0.4	—	—	—	—	—	—	0.23	1.10	0.69	—	—	Good
23	2.3	0.5	0.18	2.86	3.27	—	—	Convulsion, death	—	—	—	—	—	—
24	2.0	0.5	0.10	2.28	2.60	—	—	» »	—	—	—	—	—	—
25	2.3	0.5	—	—	—	—	—	—	0.23	1.45	0.70	—	—	Good
Average	. . .		0,18	1,36	0,98	—	—	—	0,19	0,85	0,40			

TABLE 3. Effect of Carbon Dioxide Gas on Concentration of Glutamine and Urea in the Blood and Animal after Injection of Ammonium Chloride Solution

Rabbit No.	Dose ammonium chloride g/kg	Control experiments						Experiments using carbon dioxide gas					
		Amide N of glutamine, mg %			Urea nitrogen, mg %			Amide N of glutamine, mg %			Urea nitrogen, mg %		
		before injection	after 1 hr	after 2 hr	before injection	after 1 hr	after 2 hr	before injection	after 1 hr	after 2 hr	before injection	after 1 hr	after 2 hr
6	0.1	0.70	0.85	1.03	13.0	15.5	16.6	0.75	0.90	0.91	13.0	17.0	17.0
7	0.1	0.26	0.33	0.31	9.5	10.0	12.5	0.24	0.58	0.37	9.0	9.0	12.0
8	0.1	—	—	—	13.0	12.0	13.5	—	—	—	12.0	14.0	14.0
9	0.25	0.49	1 18	0.19	20.0	24.0	25.5	0.65	1.38	0.70	15.0	22.5	21.0
10	0.25	0.73	0.99	0.16	9.0	13.0	14.0	0.62	1.37	0.51	12.5	19.5	17.5
12	0.25	0.25	0.20	0.27	9.5	8.1	7.3	0.77	1.09	0.66	9.1	13.2	11.5
14	0.25	0.73	1.57	0.97	7.7	11.5	11.1	0.65	1.49	1.14	5.8	9.3	11.2
15	0.25	0.68	1.55	0.70	10.0	12.7	12.9	0.43	0.99	—	13.9	14.6	—
16	0.25	0.67	1.84	—	14.7	23.2	—	1.05	1.93	—	15.7	20.8	—
17	0.25	0.50	0.97	—	—	—	—	0.45	1.11	—	—	—	—
18	0.25	0.62	0.81	—	8.5	19.9	—	0.69	1.11	—	11.5	13.5	—
20	0.4	—	—	—	10.0	16.0	—	—	—	—	—	—	—
21	0.4	—	—	—	—	—	—	0.85	1.41	—	16.5	20.5	—
22	0.4	—	—	—	—	—	—	0.52	0.83	0.36	13.5	18.5	20.5
23	0.5	0.57	1.34	—	14.5	19.5	—	—	—	—	—	—	—
24	0.5	0.88	1.90	—	12.0	19.0	—	—	—	—	—	—	—
25	0.5	—	—	—	—	—	—	0.90	2.05	—	16.5	25.0	—
Average . . .		0.59	1.13	0.52	11.6	15.7	14.2	0.65	1.25	0.66	12.6	16.7	15.6
Ratio to ammonia in blood		3.3	0.8	0.5	64	12	14	3.4	1.5	1.7	66	20	39

As Table 2 shows, injecting the rabbits with ammonium chloride leads to a distinct rise in the concentration of ammonia in the blood. The greater the dose of ammonium chloride, the greater the increase in the level of ammonia in the blood. If, for example, injection of 0.1 g/kg of ammonium chloride raises the level of ammonia in the blood on the average by 0.23 mg%, then on injecting 0.25 g/kg, the ammonia level in the blood rises on the average by 1.09 mg%. Injection of doses of 0.4-0.5 g/kg lead to an increase in ammonia concentration in the blood of 2-3 mg%, that is, 15-20 fold.

Corresponding to this, the condition of the experimental animals is also changed. Small doses of ammonium chloride do not cause much change in the condition of the rabbits. When higher doses of ammonium chloride (0.25 g/kg) are injected, the rabbits show an increased irritability, ataxia, convulsions, opistotonos. Injection of ammonium chloride in doses of 0.4-0.5 g/kg usually leads to death of the rabbits.

Table 2 also shows that if the rabbit, after injection of ammonium chloride, is placed for one hour in the chamber with an increased carbon dioxide content, the raised concentration of ammonia in the blood is less apparent than in the control experiments. Thus, with injection of 0.1 g/kg ammonium chloride the ammonia level in the blood under conditions of breathing carbon dioxide gas is increased on the average by 0.07 mg% (in the control experiments, as already stated, the ammonia concentration in the blood with this dosage of ammonium chloride increases by 0.23 mg%); with injection of 0.25 g/kg the ammonia concentration in the blood of the experimental animals increases by 0.69 mg% (in the controls, by 1.09 mg%). The ammonia concentration in the blood is considerably less as compared to the controls in the case of injecting fatal doses of ammonium chloride.

It must be emphasized that breathing air with an increased content of carbon dioxide gas prevents convulsions and death of the rabbits after injection of large doses of ammonium chloride. The condition of the rabbits in such a case does not as a rule differ much from the normal.

It is known that injection of ammonium chloride into an organism leads to the development of a state of acidosis. Therefore it can be assumed that the anticonvulsive effect of carbon dioxide gas in our experiments is connected with a normalization of the pH. The test experiments (Table 2) showed, however, that removal of the acidosis which develops under the influence of ammonium chloride does not occur when carbon dioxide is breathed. It seems to us that the effect can be explained by the neutralization of ammonia. In confirmation of this we also ran control experiments with injection into four rabbits of 0.25 g/kg of ammonium lactate, a salt which does not cause acidosis. Without carbon dioxide gas, the level of ammonia in the blood then rose from 0.17 to 0.97 mg% while with carbon dioxide breathing the ammonia concentration in the blood of the experimental animals rose only to 0.58 mg%.

The binding of ammonia in the animal organism occurs basically either by urea formation, or by amidation of glutamic acid. Therefore in this work along with the study of the variation of ammonia in the experimental animals we also determined the content of glutamine and urea in the blood. The results are given in Table 3, from which it is evident that the level of glutamine in the blood after injection of ammonium chloride is increased, and this effect is more marked in the experiments with use of carbon dioxide gas. When the animals are injected with 0.25 g/kg, the glutamine concentration in the blood increases on the average from 0.6 to 1.05 mg%. If, after the injection of ammonium chloride, the rabbits are placed in the chamber with the carbon dioxide gas the glutamine concentration in the blood rises on the average to 1.31 mg%.

The content of urea in the blood after ammonium chloride injection is also increased. Analysis of the data obtained on the same rabbit shows that breathing carbon dioxide gas permits a more rapid elevation of urea concentration in the blood even in the first hour after injecting the solution.

Thus, on the basis of our results we can conclude that breathing carbon dioxide gas after injection of ammonium chloride hastens the binding of the ammonia, increases the glutamine and urea formation, and prevents death of the animals after injection of large doses of ammonium chloride (0.25, 0.4 g/kg).

SUMMARY

The author studied the influence of carbon dioxide on the concentration of ammonium, glutamine, and urea in rabbit's blood. He showed that administration of ammonium chloride to rabbits in doses of 0.1 g/kg of body weight, 0.25 g/kg, 0.4 g/kg and 0.5 g/kg leads to a corresponding rise of ammonium, glutamine and urea in the blood. When rabbits are placed into a chamber containing 7-8% carbon dioxide, ammonium is neutralized with simultaneous increase in the formation of glutamine and urea. Inspiration of carbon dioxide prevents cramps and death in rabbits after administration of large doses of ammonium chloride.

LITERATURE CITED

1. E. A. Vladimirova, Byull Éksp. Biol. Med. 30, 11, 345 (1950).
2. D. L. Ferdman, in: Progress of Biological Chemistry [in Russian] (Moscow, 1950) Vol. 1, p. 216.
3. P. P. Cohen and M. Hayano, J. Biol. Chem. 166, 239 (1946).
4. P. P. Cohen and M. Hayano, J. Biol. Chem. 166, 261 (1946).
5. P. P. Cohen and M. Hayano, J. Biol. Chem. 170, 687 (1947).

6. P. P. Cohen and M. Hayano, J. Biol. Chem. 172, 405 (1948).
7. E. A. Evans and L. Slotin, J. Biol. Chem. 136, 805 (1940
8. S. Grisolia, R. H. Burris, and P. P. Cohen 191, 203 (1951).
9. H. A. Krebs and K. Henseleit, Z. physiol. Chemie 210, 33 (1932).
10. H. A. Krebs, Biochem. J. 29, 1951 (1935).
11. D. Rittenberg and H. Waelsch, J. Biol. Chem. 136, 799 (1940).

THE EFFECT OF HIGH ALTITUDE ON THE PROTEIN COMPOSITION OF HUMAN BLOOD

E. P. Smolichev

Department of Pathological Physiology (Head - Docent E. P. Smolichev),
Avicenna Stalinabad Medical Institute
(Presented by Active Member AMN SSSR S. E. Severin)
Translated from Byulletin' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 10, pp. 78-82, October, 1960
Original article submitted December 22, 1959

There are as yet no data on the effect of the complex of high altitude factors on the protein composition of the blood in humans and animals.

In cats, severe oxygen deprivation does not cause a change in blood protein composition [5]. Prolonged action of atmospheric pressure reduced to 310.8 mm Hg (4 hours daily for 21 days) in rats causes decrease in the albumin-globulin coefficient in the serum as a result of lowered albumin concentration and a considerable rise in the concentration of α_2-globulin, with decreased occurrence of a raised content of other globulin fractions in the serum [9]. "Elevation" in a pressure chamber to a "height" of 8500 meters and keeping there for three hours a day for 15 days caused in dogs a lowered content of blood γ-globulin [7]. Examination of healthy humans in mountain regions has not shown any change in blood protein composition [8] but under the same conditions physical stress causes a lowered albumin concentration and a rise in the globulin concentration [6].

The present study was designed for the purpose of explaining the effect on protein composition of blood serum of a move of people from their usual atmospheric conditions to conditions of a high altitude climate.

METHODS

We determined the protein composition of the blood in 12 young individuals of both sexes, completely healthy clinically, at Stalinabad (850 m) and under mountain conditions in Eastern Pamir at an elevation of 4200 m above sea level (during the period of the high altitude scientific expedition of the Stalinabad Medical Institute from May to October, 1958).

Blood for the study was taken in the morning, directly after sleep, on an empty stomach, from the finger into a centrifuge tube, which was at once stoppered with a tightly fitting stopper (to prevent the blood from drying, which would be particularly important at lower barometric pressures). After the blood had coagulated it was centrifuged and the total concentration of protein was determined in the serum with an RF-1 refractometer. Fractionation of the serum proteins was carried out by paper electrophoresis according to the accepted laboratory method [3]. Statistical treatment of the materials of the investigation gave the significance of the variations by a differential method [1] according to the index t.

TABLE 1. Change in Protein Concentration in Human Blood Serum (in g-percent) Before and After a Rise in Elevation.

Conditions of study / Statistical index	Before rise (850 m)	After rise (4200 m)		A month after descent
		First month	Fourth month	
M	7.3	8.2	7.7	7.8
$\sigma\pm$	0.58	0.51	0.47	0.20
P	—	<0.01	<0.05	<0.05

Note: In Tables 1 and 2 the significant difference (P) is calculated from the ratio to the initial data in Stalinabad before the ascent.

RESULTS

In Table 1 we give the statistical treatment of the results of the study of total protein concentration in blood serum.

Table 1 shows that during the first month of stay in Pamir the concentration of protein was raised significantly ($P < 0.01$). After four months at this altitude the concentration of protein in the blood fell somewhat, but remained above the starting level ($P < 0.05$). In the first month after the descent the concentration of protein in the serum continued to remain high ($P < 0.05$).

The results of the electrophoretic study of serum protein concentration are given in Table 2.

Analysis of the results of Table 2 shows that the concentration (relative and absolute) of albumin in human blood serum falls immediately after their ascent to the altitude and continues to remain truly low ($P < 0.05$) for at least a month after the descent. The concentration of α_1- and γ-globulins in humans increases immediately af-

TABLE 2. Change in Protein Composition of Human Blood Serum (in relative percent before and after ascent to altitude)

Protein fraction	Conditions of study / Statistical indexes	Before ascent (850 m)	After ascent (4200m)		A month after descent
			first month	fourth month	
Albumin	M	66.2	55.7	57.3	54.8
	σ	3.5	2.8	5.3	3.9
	P	—	<0.001	<0.001	<0.05
Globulins					
α_1	M	2.9	5.5	5.2	5.1
	σ	0.9	1.2	2.2	1.6
	P	—	<0.001	<0.02	<0.001
α_2	M	2,9	3.9	3.8	5.3
	σ	0,9	1.5	1.3	2.1
	P	—	<0.2	<0.1	<0.01
β	M	13,2	16.4	14.6	17.4
	σ	1,9	2.6	1.9	4.7
	P	—	<0.01	<0.1	<0.01
γ	M	14,8	18.5	19.1	17.4
	σ	2,6	3.6	4.9	3.2
	P	—	<0.01	<0.01	<0.05

ter the ascent and remains high after the descent. The concentration of β-globulin rises immediately after the ascent; four months later it has fallen somewhat (the difference compared to the initial value is statistically insignificant, $P < 0.1$), and after the descent it actually again rises above the initial value. The concentration of α_2-globulin is not changed by ascent to the altitude and during the period of stay, but after the descent it increases ($P < 0.01$).

The regularities of change in composition of human blood proteins which we have observed in the move from Stalinabad (850 m) to the high altitude region of Eastern Pamir (4200 m) are characterized by a decreased concentration of albumin and an increased concentration of the globulin fraction (chiefly the γ-, α_1-, and β-globulins) with increased concentration of total protein in the serum.

The disturbance in blood protein composition is found during the four month stay of the individuals under high altitude conditions and is kept after the descent (a period of one month observation). The length of the disturbance does not permit explaining it only by a concentration of the blood or a redistribution of proteins between blood and extravascular intercellular tissue fluids. It is possible that these factors have some effect only at the beginning of the change in protein composition of the blood. The observed disturbance in protein composition of the blood has a nonspecific character. The organism reacts to any extreme and unusual stimulus by lowering the albumin content and increasing the content of globulins in the serum [2, 10]. Such a reaction of the protein composition of the blood is considered as a humoral effect in the first phase of the Selye adaptation syndrome [10]. Actually, the move of humans from ordinary atmospheric pressure (680 mm) to conditions of decreased atmospheric pressure (450 mm) and the return have been considered a stress which causes an internal functional reorganization of the whole physiological system of the organism. Evidently, under these conditions, as usual there is a weakening of albumin synthesis and a compensatory strengthening of synthesis of the globulins.

One of the most important functions of the plasma proteins is the maintainance of a constant oncotic pressure of the blood, and hence an equal distribution of the water between the vessels and the extravascular spaces. In this connection it is of interest to explain the change in oncotic pressure of the blood under high altitude conditions. In Table 3 we give values for the oncotic pressure of the blood calculated on the basis of determination of the concentration of total protein, albumin, and globulins.

Table 3 shows that only in the first month after ascent to the altitude was the oncotic pressure of the blood above the initial value. With further stay at the altitude it became equal to the original and did not change with descent. In the first month of stay at the altitude the total protein concentration in human serum rose on the average by 0.9% (albumin concentration decreased by 0.2%, but the globulins increased by 1.1%). At the end of the stay at the altitude the concentration of total protein in the serum fell somewhat (increase above the initial value of 0.4%). However, with this there was a change in ratio between concentration of albumin and total globulins (albumin concentration 0.4% below initial level, and globulins 0.8% above initial level). As a

TABLE 3. Change in Oncotic Pressure of Human Blood Serum (in cm of water)

Index	Before ascent (850 m)	After ascent (4200 m)		A month after descent
		first month	fourth month	
Albumin (g%)	4.8	4.6	4.4	4.2
Globulins (g%)	2.5	3.6	3.3	3.6
ΔA (g%)	—	— 0.2	— 0.4	— 0.6
$\Delta \Gamma$ (g%)	—	+ 1.1	+ 0.8	+ 1.1
Albumin-globulin coefficient	1.9	1.3	1.3	1.2
Oncotic pressure				
I	34.8	39.8	36.5	36.0
II	33.6	36.9	33.9	33.5
III	32.2	32.8	31.2	30.5
M	33.5	36.5	33.8	33.3

Note: 1. ΔA and $\Delta \Gamma$ are values for variation of albumin and globulin concentrations from the initial values before the ascent. 2. Oncotic pressure of the blood (OBP) in mm water is calculated from formulas of [4]: I. OBP=C(21.4 + 5.9A); II. OBP=C(21.1 + 5.2A); III. OBP= (3.56 + Γ)/0.06, where C, A, and Γ are the concentrations in gram-percent of total protein, albumin, and globulins. 3. M is the arithmetical average of oncotic pressure from calculation by the three equations.

result there is a normalization of the value for oncotic pressure. Almost the same ratio is maintained in the month after descent. Hence, in the initial period of stay at the altitude the increased oncotic pressure of the blood can be explained by the hypercompensatory reaction of the organism to lessened albumin concentration, consisting in excess accumulation of globulins in the blood ($\Delta\Gamma/\Delta A$=5.5). With further increase in globulin concentration the albumin concentration begins to correspond ($\Delta\Gamma/\Delta A \simeq 2$) and the oncotic pressure is normalized.

Thus, we can present a mechanism for the normalization of oncotic pressure in the adaptation of individuals to high altitude conditions in spite of maintenance of a disturbed protein composition of the blood.*

SUMMARY

Twelve healthy young individuals had the protein composition of their blood determined in Stalinabad (850 m) and during their sojourn in the mountain of East Pamir between the months of May and October, 1958, at the altitude of 4200 m.

During the first month of sojourn at high altitude, the total concentration of protein went up, while towards the end of the 4 month period it dropped somewhat, but still remained above the initial level. The relative and the absolute albumin content in the blood serum dropped immediately after the ascent and remained low for a month after descent from 4200 m to 850 m. The figures for α_1-, β- and γ-globulins rose immediately after ascent. During the 4 month stay at high altitude, the α_1- and γ-globulins went back to normal. During the first month after descent, figures for α_1-, β- and γ-globulins were higher than the initial levels; α_2-globulin went up only after descent. Oncotic pressure of blood rose during the first month after ascent, then returned to normal at the expense of increased concentration of the globulin fraction, which compensates for the decreased albumin content in blood serum.

LITERATURE CITED

1. I. A. Oivin, in: Materials on the Pathology of Blood Proteins and Disturbance of Vascular Permeability [in Russian] (Stalinabad, 1959) p. 149.
2. I. A. Oivin, V. I. Oivin, and E. P. Smolichev, in: Materials on the Pathology of Blood Proteins and Disturbance of Vascular Permeability [in Russian] (Stalinabad, 1959) p. 5.
3. E. P. Smolichev, in: Materials on Inflammation and Pathology of Vascular Permeability [in Russian] (Stalinabad, 1956) No. 3, p. 237.
4. Z. Autio, Körkkäinen, et al., Ann. Med. Exptl. et Biol. fenniqe 35, 209 (1957).
5. R. Fisher and F. Wullen. Z. ges. exper. Med. 130, 538 (1959).
6. W. Heinen, J. Czaja, H. Loosen, et al., cited by F. Wuhrmann and C. Wunderly.
7. J. Malmejac, S. Cruck and G. Neverre, Med. aéro. 5, 135 (1950).
8. A. Muralt and B. Natter cited by Wuhrmann F., Wunderly C.
9. S. J. Piliero, Arch Biochem. a Biophys., Vol. 5, No. 1, 1958, p. 248.
10. F. Wuhramann and C. Wunderly Die Bluteiweisskörper des Menschen. Basel, S. 315, 1957.

* In conclusion, I consider it my duty to express thanks to Prof. I. A. Oivin for consultation in this work.

MITOGENETIC RADIATION OF THE NEUROMUSCULAR SYSTEM AS A METHOD OF ANALYZING ITS MOLECULAR SUBSTRATE

Communication II. On the Application of the Concept of Molecular Substrate Regulation in the Resting Muscle

A. A. Gurvich

Mitogenesis Office of the Institute of Normal and Pathological Physiology
(Dir.-Active Member AMN SSSR V. N. Chernigovskii),
AMN SSSR, Moscow
(Presented by Active Member AMN SSSR V. N. Chernigovskii)
Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 10, pp. 82-86, October, 1960
Original article submitted June 30, 1958

Investigating the resting state of the neuromuscular system as an "active background" in the introduction to our first report [1], we related this idea to the concept of interacting processes, occurring in the molecular substrate of the system. The resulting state is characterized by uninterrupted molecular regulation, maintained in an unequilibrated condition [2, 3, 4, 6]. Thus, we may state that at every given moment the state of each individual system, mentally separated from the "whole," is, to a considerable degree, "tied-up" with the actions of the other systems.*

But the concept of unequilibrated molecular regulation is, by its nature, dynamic. The order of the molecular substrate is uninterruptedly and incompletely broken down and built up again; with slight exaggeration we can speak of this as the uninterrupted renewal of the "organization" of the substrate, or, drawing on a more common biological concept, we may describe it as an uninterrupted "regulation." The principle of "regulation" of the substrate in the resting stage may be regarded, then, as a completely tangible question, and its analysisserves as the goal of this and subsequent reports.

The muscles of mammals and cold-blooded animals in situ are subjects on which a study of the molecular substrate, from the aforementioned point of view, is not only completely accessible but also presents special interest. A number of signs make it probable that the "resting" radiation of the nervous and muscular systems represents a "physiological" degradation radiation [1, 2, 3, 6].

A comparison of the relative radiation intensity in the resting state and with degradation caused by chilling, and a study of the spectra under these same conditions, made it possible to draw several conclusions on the regulation of the muscle substrate in the resting state.

EXPERIMENTAL METHOD AND RESULTS

Total radiation of the calf muscle, M. gastrocnemius, in the frog under different temperature conditions. The basic purpose of these experiments consisted of comparing the radiation intensity of a muscle at normal temperature (15-16°) and with significant chilling of its surface (to 2-3°). The conditions were standardized as much as possible: the radiating surface of the upper part of the muscle was constant–10-12 mm^2, and the distance from the detector was also kept the same–10 mm. The experiments were carried out in the Fall and Winter on Rana temporaria and Rana ridibunda (males). The muscles were chilled by intense streams of cold physiological saline or by direct insertion of a small thin icicle of frozen physiological saline into the muscle.† In both modifications the chilling was begun immediately before the exposures. The intensity of radiation was determined according to the relation Jt=const.

Thus, judging from the marked drop in the plate exposure, the radiation of the muscle following chilling was approximately 3 times more intense than the radiation at 15-16°.

On the basis of these data the following general conclusion may be made. A resting muscle exists at a high energy level; the energy output in this case (in the form of radiation, for example) is very small, but it is easily elevated by artificial intervention, e.g., chilling, which is evidence of its high potential energy. This latter fact also shows that the energy state of the muscle is maximal-

* In the strict sense, one should speak of the "interlaced" character of the substrate in each microvolume of each system.

† The physiological saline was cleared for the radiation.

TABLE 1. Radiation of the Calf Muscle in the Resting State (in percentages)*

Exposure (in seconds)	At normal temperature (15-16°)	With chilling (to 2-3°)
3	-7, -8,6	31 ± 8***, 10, 42, 66, 35
5	4, 6	—
8	35 ± 12**, 63,52,33,39,60,65	—

*Here and in the following table the effect in % is calculated according to the formula $\frac{O-K}{K} \cdot 100$, where O is the experimental value and K is the control.

**The probable error, calculated for the average trials (at the height of the effects), illustrates their reliability.

***Footnote indicated but omitted in Russian - Publisher.

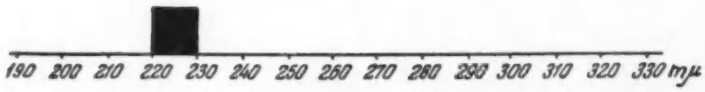


Fig. 1. Radiation spectrum of the calf muscle of the frog in the resting state.

ly labile, i.e., unequilibrated, and, in addition, makes the converse inference probable: doubtlessly, the uninterrupted utilization of energy, occurring in the physiological resting state, is accomplished very economically. In such a general form this inference is not directly linked to the concept of the unequilibrated molecular order, but any attempt to ground the inference more strongly leads to this concept, since spatial propinquity of the molecules at the moment of their excitation is the most adequate mechanism for the economic accomplishment of a series of processes.

Spectral analysis of muscle radiations. The value of mitogenetic spectral analysis is especially clear in work on the organism as a whole. By studying the spectra of degradation radiation and of radiation from the system in the resting state we were able to come to a certain opinion on the "preciseness" or on the "degree" of reproducibility of the structural status of the substrate. Unquestionably, the barren appearance of the spectra, i.e., the limited number of spectral bands, is directly related to the monotypic nature of the molecular arrangement—that is, the more or less strict reproducibility and recurrence of the molecules' orientation and deformation. In these experiments we employed a relatively crude spectral analysis of the radiation, but, in so doing, were able to cover a number of spectral areas simultaneously, which is important in studies of the physiological state in situ.

We studied the spectrum of the calf muscle in the resting state at both normal temperature (15-16°) [5] and during the cooling of the muscle to 3-4°. As in the case of the previous experiments, we laid bare the standard surface in the upper portion of the muscle, and the frog was secured in such a fashion that the bared segment was situated in front of the inlet aperture of the spectrograph. Metal discs (so called templets) were alternately inserted in the focal plane of the outlet aperture. These discs bore sets of windows calculated to lie in such a way that the entire effective range of mitogenetic radiation (1900-3200 A) was analyzed in regions of 100 A.‡ The spectral maximum for the resting state radiation was observed at 8-10 seconds. With cooling the plate exposure was decreased to 5-6 seconds.

The results of the experiments showed that the effective spectral range was the same for both modifications of the investigation. The spectrum was very limited. A clearly manifested radiation intensity was observed only in the 2200-2300 A range (Table 2). The entire band of wave lengths to the right and left of this segment, even examined with increased exposure, did not produce a positive reading, i.e., it could be regarded as a significantly reduced background in comparison to the maximum segment.

A radiation spectrum as unusual as this requires further study. It was necessary to clarify whether such a barren spectrum was characteristic for the resting radiation of muscles in general or only for certain types of muscles. From this point of view, we studied the spectra

‡An independent biodetector was placed against each window.

TABLE 2. Radiation Spectrum of the Calf Muscle (in percentage)

Wave length (in A)	At normal temperature (15–16 deg)			With cooling (to 3–4 deg)	
	I	I	II	III	III
1900—2000	—7	5	—6	—2	5
2000—2100	4	—	—5	—3	8
2100—2200	—	—	5	12	—
2200—2300	34	48	45	53	48
2300—2400	—8	—	—5	11	8
2400—2500	—	—	2	—4	0
2500—2600	10	—2	—11	16	6
2600—2700	—8	—	3	—11	10
2700—2800	—	—	—2	4	—
2800—2900	—8	0	11	10	5
2900—3000	—2	—	10	—3	—1
3000—3100	—	—	5	13	—
3100—3200	—3	10	—6	—10	11

Note: I–8-second exposure; II–16-second exposure; III–5-second exposure (Fig. 1).

It is naturally necessary to take into consideration that ranges of 100 A yield arbitrary boundaries for the spectral maximum. For example, breaking up the 2200-2300 A range into 20 A bands showed that the maximum effectiveness was situated in the first 60 A, and that the intensity fell in the last 40 A. In the investigations necessary for our problem, however, such arbitrary division into 100 A bands is still sufficient.

of the resting and degradation radiations from the m. semimembranosis and the considerably smaller m. tarsalis anticus.

The data obtained affords the opportunity of making the following conclusion. The spectrum of the semimembranosis muscle in the resting state–at both normal and decreased temperatures–is principally close to the spectrum of the calf muscle. An intense maximum, appearing when the exposure was 8-10 sec, was also characteristic of its spectrum. But differences did exist: the maximum radiation occupied a different spectral range–2000-2100 A; in addition, when the exposure was approximately doubled weaker spectral bands appeared. The most intense of these was the range 2500-2600A, and the considerably weaker ranges 2200-2300 A and 2700-2800 A (Fig. 2).

Thus, in comparison with the calf muscle the spectrum of the semimembranosis muscle is less limited.

The spectrum of the m. tibialis anticus differed from the spectra of both the calf muscle and the semimembranosis. The intense radiation (approximately of the same order as for the other two muscles) fell in the range 1900-2000 A; in addition, a wide swatch of the wave length spectrum, 2800-3200 A, was unquestionably active (Fig. 3).

It is necessary to take note of the fact that, in contradistinction to the two previous muscles, whose radiations were constant at different times of the year, the radiation of the anterior tibial muscle was more intense in the spring than in the fall and winter (the latter two seasons were when these data were obtained).

The following general conclusions are possible from a comparison of the spectra. Muscles at rest possess a clearly expressed "spectral individuality". This concept

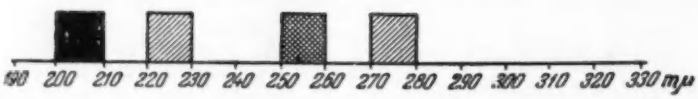


Fig. 2. Radiation spectrum of the semimembranosis muscle of the frog in the resting state. The depth of shading of the bar is proportional to the intensity of the radiation.

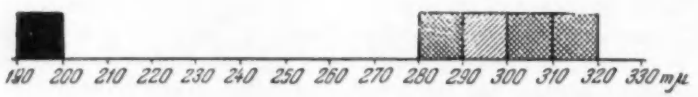


Fig. 3. Radiation spectrum of the m. tibialis anticus of the frog in the resting state. The depth of shading of the bar is proportional to the intensity of the radiation.

may be regarded as a rule, since there is no basis for believing that the observed phenomenon is limited to the muscles studied. The highly delimited spectrum strikingly manifested in the analysis of the calf muscle did not occupy a wide portion of the wave length band. The possibility that this limitedness is more marked in the coarser muscles has not been excluded. This question, which we regard as highly important, continues to be studied. But as far as the calf muscle is concerned, we can freely state, in all cases, the opinion advanced above, i.e., that the monotypic nature of the molecular substrate structure of this muscle is more impressive than in other muscles.

These conclusions make it possible to go somewhat further and formulate the following inferences. The resting state of the different muscles, uniform from a functional point of view, is attained by dint of the different variations in their molecular substrates. This can mean only one thing: in order to appraise the resting state of a muscle one can never be limited to describing it as a localized system; it is absolutely necessary to bring in the concept of the "adequacy" of its state as pertains to the state of another system, perhaps even isolated from it but nevertheless interdependent upon it. The resting state is itself a functional manifestation of such adequacy, but the means by which it is attained may be different, and, correspondingly, the resulting natures of the molecular substrates may also differ from one another.

Working with the idea of the molecular substrate, we start from the statistical molecular multitude and characterize any state from the point of view of statistical laws, i.e., we speak of their probability. But in this case it is very important to remember the dynamic facet of all phenomena and to regard the probable state of the substrate, not as the manifestation of a stable condition, but rather as a reflection of the fact that the particular "type" or "character" of the interacting processes arises more often than the other variants.

In other words, we see our main problem specifically in analyzing the continuous accomplishment of a given or many given states. From this viewpoint, we are studying the principle of substrate regulation in muscles at rest, and we are attaching principal importance to the application of this concept in the study of phenomena observed in the nervous and muscular systems.

SUMMARY

Data are presented which permit regarding the mitogenetic muscle radiation in vivo at the state of rest as a "physiological" degradational radiation. The radiation spectra of the frog muscles (m. gastrocnemius, m. semimembranosus, and m. tarsalis anticus) differ in the quantity and distribution of the active bands. The least amount of the latter is present in the spectrum of gastrocnemius muscle, whereas m. tarsalis ant. contains the largest quantity of the bands. The regulation of the muscle's substrate at the state of rest is envisaged on the basis of these results.

LITERATURE CITED

1. A. A. Gurvich, Byull. Éksp. Biol. Med. 49, No. 5 (1960).••
2. A. Gurvich and L. Gurvich, in: Mitogenetic Radiation [in Russian] (Moscow, 1945).
3. A. Gurvich and L. Gurvich, in: Preface to the Studies on Mitogenesis [in Russian] (Moscow, 1948).
4. V. F. Eremeev, Byull. Éksp. Biol. Med. 45, 6, 95 (1958).••
5. M. A. Lipkind, Byull. Éksp. Biol. Med. 45, 6, 65 (1958).••
6. A. G. Gurwitsch and L. D. Gurwitsch, Die mitogenetische Strahlung (Jena, 1959).

•• Original Russian pagination. See C. B. translation.

AGE SENSITIVITY TO BARBITURATES AND ITS DEPENDENCE ON THE DEVELOPMENT OF THE HYPOPHYSEAL - ADRENAL SYSTEM

I. V. Markova

Department of Pharmacology (Head - V. M. Karasik, Active Member of the AMN SSSR) of the Leningrad Institute of Pediatric Medicine
(Presented by V. N. Chernigovskii, Active Member of the AMN SSSR)
Translated from Byulleten' Eksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 87-91, October, 1960
Original article submitted December 22, 1959

According to the literature [5] and to our own data [2,3], newborn animals and those at early stages of postnatal development are considerably more sensitive than mature animals to narcotics. Since this greater sensitivity of very young animals has been observed with the use of both medinal [barbital sodium] and ether, narcotics which are not broken down in the organism, it cannot, as some authors believe [5], be held to depend on the rate of the drug's detoxication in the organism.

According to the literary data [11, 14], extirpation of the adrenal glands prolongs and deepens narcosis in rats and mice. This prolongation is due to the higher barbiturate content in the brain of adrenalectomized animals [14].

Because the function of the hypophyseal-adrenal system is insufficient during the early stages of postnatal development in rats and other animals [7,9,12], these very young animals can be compared with mature animals from which the adrenohypophysis has been removed.

This led us to propose that the the higher sensitivity of newborn animals to barbiturates might be due to their underdeveloped hypophyseal-adrenal system. This hypothesis could be confirmed by experimentally determining whether the removal of the adrenal glands from baby rats (in which the hypophyseal-adrenal system is not yet fully developed) would have a lesser effect on the duration of the narcotic influence than in the case of mature rats.

According to the data of Hungarian authors [12], the hypothalamus does not begin its regulation of the hypophyseal-adrenal system function in rats until the animals are two weeks old; therefore, one can propose that, from this time on, gluco-corticoids, hitherto not secreted in physiologically adequate quantities, increase the resistance of the vascular wall, thereby inhibiting penetration of the barbiturates into the central nervous system and, in this way, decreasing their narcotic effect. The greater permeability of the blood-brain barrier in newborn rats is attested to in the works of L. S. Shtern [6], Bakay [8], et al.

In this work, we determined: 1) the effect of adrenalectomy on the narcotic effect of hexenal and 2) the content of medinal in the brain of rats of various ages.

EXPERIMENTAL METHODS

The first series of experiments were carried out in 75 rats from three age groups (adult males, six-week-old males and females and month-old males and females from the same nursery). Adrenalectomy was performed from the dorsal aspect. To keep the animals alive, we administered cortisone (Roussel) before the operation in a dose of 30-50 γ/1 g of animal weight. After the operation, the animals were fed only water and a 0.9% sodium chloride solution. After 1-5 days, we again determined the duration of the lateral (reclining) posture induced in the rats by hexenal, intraperitoneally or subcutaneously injected in a dose of 80-150 γ/g and compared this with the duration of the lateral posture in the control rats. The individual animals were usually given the barbiturate injections only once every 5 days. The observation period was limited to 2-3 weeks by the fact that most of the adrenalectomized animals died within this period.

A second series of experiments was performed on 250 rats from one nursery. In the three-week-old or younger age groups, we used both male and female rats in the experiment; the older rats used were all females. The rats of each age group (except for the mature age group) were all used in the experiment the same day. Every rat was intraperitoneally injected with a uniform dose of medinal (200 γ/g each). Medinal was chosen for two reasons: one, because it does not break down in the organism, and two, because the medinal dose inducing the lateral posture in mature rats can be tolerated by newborn rats. The rats were decapitated at fixed time intervals (10, 20, 40, 60 and 150 min) after the medinal injection, and the medinal content of the brain was determined according to the method described by

TABLE 1. Medinal Content in Brain of Experimental Rats of Different Ages after Its Intraperitoneal Injection

Age of rats	Amount of medinal (in γ/100 mg of dry brain weight)				
	Time after intraperitoneal medinal injection				
	10 min	20 min	40 min	60 min	150 min
Newborn	117.4±4.4	184.2±8.4	165.6±2.4	167.2±11.6	170.2±11.3
1 week	168.5±10.8	171.4±7.7	195.3±13.3	253.3±22.4	229.4±32.5
2 weeks	49.6±3.1	130.2±7.7	142.6±7.1	107.5±4.6	90.2±4.4
3 weeks	91.3±1.17	108.4±3.9	109.9±5.7	111.9±4.5	132.7±1.7
4 weeks	60.1±6.5	114.5±3.2	98.0±5.4	103.4±2.3	87.4±4.2
1 1/2 mos	38.2±6.7	128.8±4.3	147.6±9.0	94.5±2.1	71.2±3.7
Adult	66.4±8.7	74.5±6.8	112.6±9.1	99.3±10.0	66.5±8.9

Goldschmidt and co-workers [13]. Five animals were sacrificed for each determination of the medinal content of the brain. In the experiments with newborn animals, the brains of two rats were used for each determination. The medinal was extracted from the cerebral hemispheres (barbiturates are known to disperse rather evenly in the brain [16]). Since the water content of the brain varies in rats of different ages [10], one would expect different quantities of medinal to be extracted. However, the addition of medinal to and its subsequent extraction from brain homogenates taken from newborn and adult rats showed that 80% of the medinal added was extracted.

EXPERIMENTAL RESULTS

Fig. 1 illustrates the data from the experiments with extirpation of the adrenal glands, showing the change in the ratio of the average duration of the lateral posture in the experimental rats (M_1) to that in the control (M_2). It is evident from the graph that removal of the adrenal glands sharply prolonged hexenal narcosis in the case of adult males; 14 days after the operation, the duration of narcosis was 6½ times longer than the control. According to the literary data [11, 19], it has been observed to become 20-360 times longer. In six-week-old rats, extirpation of the adrenal glands had less influence on the duration of the lateral posture, and this influence was more marked in the males than in the females. In month-old females, only a slight (statistically unreliable) prolongation of the lateral posture was observed in some of the experiments (on the 5th and 15th days) after adrenalectomy. In month-old males, no changes in the duration of the lateral posture were observed after extirpation of the adrenal glands.

Table 1 and Fig. 2 illustrate the medinal content in the brains of rats of different ages. The data cited indicate that the greatest amounts of medinal were found in the brains of newborn and week-old rats. The medinal content in the brains of rats two or more weeks old was smaller, and by the 3rd-4th weeks approximated that found in adult rats. In almost all the age groups, the maximal content of medinal in the brain was observed 20-40 min after its injection. The week-old group of rats were, however, an exception, as the

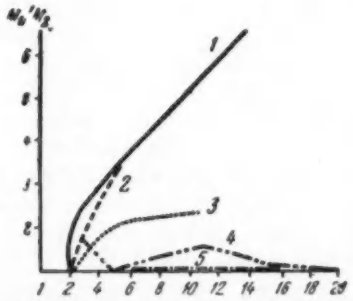


Fig. 1. Change in duration of the lateral posture induced by hexenal in adrenalectomized rats of different ages. 1) Adult males; 2) six-week old males; 3) six-week old females; 4) month-old females; 5) month-old males.

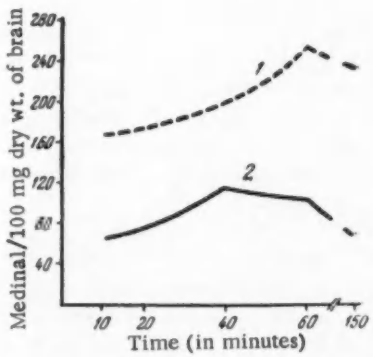


Fig. 2. Medinal content in brains of experimental rats after intraperitoneal injection of 200γ medinal per 1 g of weight. 1) Week-old rats; 2) adults.

TABLE 2. Lateral Posture in Rats of Different Ages with Intraperitoneal Injection of Medinal

Age of rats	Ratio of number of rats in lateral posture (numerator) to number of rats used in expt. (denominator)				
	Time after medinal injection				
	10 min	20 min	40 min	60 min	150 min
Newborn	8/8	10/10	10/10	10/10	10/10
1 week	4/4	8/8	8/8	8/8	4/4
2 weeks	4/5	5/5	5/5	4/5	5/5
3 weeks	0/5	4/5	5/5	4/5	5/5
4 weeks	0/5	2/5	5/5	5/5	5/5
1 1/2 mos	0/5	0/5	4/5	5/5	5/5
Adult	0/5	2/5	10/5	5/5	3/5

medinal content of their brains continued to increase for one hour.

The speed with which the rats went into the lateral posture corresponded to the rate of medinal accumulation in the brain. Table 2 gives the number of animals in each group found in the lateral posture. Up to the age of two weeks, as Table 2 shows, the lateral posture developed very quickly (within 10 min) and continued throughout the 2½ hours of observation. The lateral posture was slower to occur in rats aged three weeks or more, and narcosis ceased in some of the adult rats towards the end of the observation period.

According to the literary data, the hypophyseal-adrenal system does not mature in rats until the animal is 2-3 weeks old [7,9]. This opinion was based on tests: decrease in the ascorbic acid content of the adrenal glands or in the blood eosinophils in response to stimulations inducing a condition of stress. The results of our experiments, however, indicate that the function of the hypophyseal-adrenal system is still insufficient in rats a month old, as at this age, removal of the adrenal glands had hardly any influence on the duration of the lateral position induced in the rats by hexenal. The reason for the variance of our observations with the literary data may be that the various systems and organs of the body acquire the ability to react to secreted corticoids at different stages in the animal's development, the peripheral organs (the hematopoietic organs and the adrenal glands) acquiring this ability earlier than does the central nervous system.

Now, how can the maturation of the hypophyseal-adrenal system affect the reactivity of the developing organism to narcotics, in particular to barbiturates?

Gluco-corticoids are known to raise the tonus of the central nervous system. This is evinced by the increase in the "electroconvulsive threshold" of experimental animals and in the greater excitability of the central nervous system in people who have taken cortisone or ACTH for a long time [19]. Therefore, because of the deficiency of gluco-corticoids in very young animals, it is possible that the tonus of their central nervous system is comparatively low, and this too would promote barbiturate narcosis.

Gluco-corticoids are also known to strengthen the resistance of the vascular walls [4, 17], and a deficiency of these substances could increase the penetration of various substances, barbiturates included, into the central nervous system.

The fact that we found a greater amount of medinal in the brains of the very young experimental rats confirms the hypothesis of its greater penetration into the brain tissue. The medinal content is lower when the hypophyseal-adrenal system of the rats is mature [12]. At about this time, the sensitivity of the young rats to medinal becomes equal to that of adult rats [2,3]. Also at this time, ACTH injections begin to have an abbreviating effect on barbiturate narcosis [1].

Hexenal is known to penetrate the central nervous system more strongly and quickly than medinal [15]. It is only natural, therefore, that hexenal penetration is not affected as soon as that of medinal by the increase in vascular wall resistance. A considerably greater degree of development is required to inhibit hexenal penetration into the brain of a growing animal.

Yugoslav researchers have recently published an observation [18] to the effect that the hypophyseal-adrenal system of newborn animals, because of the transition to life under new conditions, is in a state of hyperfunction and therefore cannot react to additional stress. Our data do not favor this hypothesis. If the hypophyseal-adrenal ystem of newborn animals were in a state of hyperfunction, their sensitivity to narcotics should be less than that of adults; in the latter, injections of ACTH, which intensifies the function of the adrenal cortex, diminish the narcotic effect of barbiturates [19]. Newborn animals, however, are extraordinarily sensitive to narcotics [2, 3, 5]. If the hypophyseal-adrenal system were in a state of hyperfunction, extirpation of the adrenal glands from infant animals would acutely intensify and prolong narcosis. Even in animals a month

old, however, adrenalectomy does not affect the narcotic action of barbiturates.

SUMMARY

Removal of the adrenals in adult rats markedly prolongs the lateral posture induced by the intraperitoneal injection of hexenal; in one-month-old rats, the removal of the adrenals exerts no such effect. After the intraperitoneal injection of 200 γ of medinal per g of body weight, spectrography revealed a high medinal content in the brains of the newly born and one-week-old rats; in two-week-old rats, the content of medinal in the brain decreases, and after 3 – 4 weeks, it reaches the level observed in adult rats. The greater penetration of medinal into the brain of very young rats is believed to be due to the underdevelopment of the hypothalamohypophyseal-adrenal system. These data shed light on the earlier investigations: after three weeks of life the sensitivity of rats to medinal becomes equal to the tolerance of the adult animals, and from the same age, injections of ACTH begin to shorten the duration of barbiturate narcosis.

LITERATURE CITED

1. I. V. Markova, Byull. Éksp. Biol. Med. 47, 3, 57 (1959). *
2. I. V. Markova, in: Transactions of the IX Convention of the All-Union Society of Physiologists, Biochemists and Pharmacologists [in Russian] (1959), Vol. 2, p. 172.
3. I. V. Markova, Fiziol. Zhur. 68, 12, 1485 (1959).
4. G. L. Mednik, Problemy Endokrinol. i Gormonoterap. 3, 2, 40 (1957).
5. T. N. Reshtnikova, Author's Abstract of Candidate's Dissertation: The Features of Barbiturates' Effect on Young Animals [in Russian] (Tashkent, 1956).
6. L. S. Shtern, in: The Blood-Brain Barrier [in Russian] (Moscow, 1935).
7. I. A. Eskin, Pat. Fiziol. i Éksp. Terap. 1, 4, 10 (1957).
8. L. Bakay, in: Blood-Brain Barrier (Springfield, Illinois, USA, 1956)
9. H. W. Deane, in Collection: Adrenal Function in Infants and Children. A. symposium N. J. (London, 1954) No. 4.
10. H. H. Donaldson, J. Compar. Neurology and Psychol. 26, 2, 119 (1910).
11. F. Eichholtz, R. Hotovv, E. Collischonn and H. Kauer, Arch. Exp. Path. and Pharmakol. 207, 5, 397 (1949).
12. E. Endröczi, J. Szalay and K. Lissak, Endokrinologie 34, 5 – 6, 331 (1957).
13. S. Goldschmidt, W. Lamprecht and E. Heimreich, Z. f. Physiol. Chemie 292, 3 – 5, 125 (1953).
14. A. Komiya and K. Shibata, J. Pharm. and Exp. Therap. 117, 1, 68 (1956).
15. L. C. Mark, J. J. Burns, L. Brand, C. I. Campomanes, N. Trousof, E. M. Papper and B. B. Brodie, J. Pharm. and Exp. Therap. 123, 1, 70 (1958).
16. L. C. Mark, J. J. Burns, C. I. Campomanes, S. H. Ngai, N. Trousof, E. M. Papper and B. B. Brodie, J. Pharm. and Exp. Therap. 119, 1, 35 (1957).
17. V. Menkin, Am. J. Physiol. 129, 3, 691 (1940).
18. K. Milkovic and S. Milkovic, Endokrinologie, 37, 5 – 6, 301 (1959).
19. D. M. Woodbury, Pharmacological Reviews 110, 2, 275 (1958).

* Original Russian pagination. See C. B. translation.

SENSITIZING PROPERTIES OF THE PARENCHYMATOUS ORGANS OF KITTENS AND WHITE MICE INFECTED WITH DYSENTERY BACILLI AND STREPTOCOCCI

M. V. Zemskov

Department of Microbiology (Head-Prof. M. V. Zemskov)
of the Voronezh Medical Institute
(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 92-97, October, 1960
Original article submitted October 3, 1959

Information regarding the sensitizing properties of the internal organs and the formation of autoantigens and corresponding autoantibodies in certain diseases and pathological states may be found in papers by several authors [1-6].

In the present communication we report the results of investigations, carried out jointly with S. A. Ignat'eva, E. D. Zhuravleva, N. K. Bondarenko and V. P. Morozova, and concerned with the study of the sensitizing properties of the parenchymatous organs of kittens and white mice infected with dysentery bacilli and streptococci.

EXPERIMENTAL METHOD

The experiments were conducted by the method published previously [2]. As a supplementary procedure the final desensitization was also performed in relation to a culture of the infecting agent, which was also injected into the heart in a dose of $1\text{-}2 \times 10^9$ bacterial cells in a volume of 1 ml. In no case (in more than 100 experiments) did guinea pigs, sensitized with infected organs, react to a culture of dysentery bacilli and streptococci.

The only exception was formed by experiments with the organs of mice inoculated with brucellosis and tularemia vaccines, in which a shock reaction was observed to injection of the latter. Desensitization with saline extracts of organs in the experiments to be described was carried out in two stages: a dose of 2 ml was given subcutaneously, followed 24 hours later by doses of 0.2 to 0.8 ml into the heart. In order to render the animals completely desensitized, two or, more rarely, three injections had to be given. We give below an extract from the records of experiment no. 3 on June 20, 1958.

Guinea pig no. 7. On June 20, 1958, 2 ml of a saline extract (19.2‰ protein) of the kidneys of a kitten, killed with ether on the seventh day after infection with Shigella flexneri, was injected subcutaneously.

July 10, 1958. 10 A. M. 2 ml of a saline extract of the kidneys of a healthy kitten injected. 12 noon. Reaction +.

July 11, 1958. 0.2 ml of a saline extract from the kidneys of a healthy kitten injected into the heart. No reaction. 12 noon. 0.8 ml of the same material injected into the heart. No reaction. 2 P. M. 2×10^9 bacterial cells (1 ml) of a 24-hour culture of Shigella flexneri on agar injected into the heart. No reaction. 4 P.M. 0.8 ml of a saline extract of the kidneys of a kitten infected with dysentery and killed with ether on the seventh day, injected into the heart. 4:12 P.M. Reaction ++++.

Necropsy: emphysema of the lungs; small quantity of serous fluid in the pericardial cavity; intestinal mucosa hyperemic.

Very often after the injection of 0.2 ml of the desensitizing material into the heart a reaction of + or ++, or even +++, was observed. In this case the routine dose was 0.3 or 0.4 ml, and the last dose 0.6 or 0.8 ml.

EXPERIMENTAL RESULTS

The results of the experiments with the organs of white mice and kittens infected with dysentery bacilli are shown in Table 1, in which the concluding anaphylactic reaction is indicated.

It will be seen from Table 1 that of the 18 guinea pigs sensitized to the liver of infected mice and kittens 16 reacted to the assaulting dose by a varying degree of shock, including 5 guinea pigs whose reaction was ++++; correspondingly, of the 12 guinea pigs sensitized to pathological spleen, a shock reaction was given by 9, in 4 of which its intensity was ++++; finally, of the 13 guinea pigs sensitized to tissue from the kidneys, a shock reaction was given by 10, including 4 to the degree ++++. Altogether, of 43 experimental guinea pigs, 35 reacted by shock and 8 gave a negative reaction.

TABLE 1. Results of Experiments with Organs of Mice and Kittens Infected with Shigella flexneri

Sensitizing material	No. of guinea pigs		Concluding reaction to sensitizing material and number of guinea pigs				
	Controls	Desensitized	−	+	++	+++	++++
Liver of mice:							
24 hours after infection		4	1	2	0	1	0
healthy		4	1	3	0	0	0
	3	0	3	0	0	0	0
Liver of mice:							
5 days after infection		7	0	2	1	3	1
healthy		5	2	3	0	0	0
	5	0	5	0	0	0	0
Spleen of mice:							
5 days after infection		5	1	0	1	0	3
healthy		6	5	1	0	0	0
	5	0	5	0	0	0	0
Kidney of mice:							
5 days after infection		6	0	4	0	0	2
healthy		4	1	3	0	0	0
	6	0	6	0	0	0	0
Liver of kittens:							
7 days after infection		7	1	1	1	0	4
healthy		3	1	2	0	0	0
	3	0	3	0	0	0	0
Spleen of kittens:							
7 days after infection		7	2	3	0	1	1
healthy		3	1	1	0	1	0
	3	0	3	0	0	0	0

(Concluded on next page)

Legend: + scratches nose with paws; ++ the same plus sneezing and shaggy fur; +++ the same plus cough, all manifestations more pronounced; ++++ excitation, jumping about, fits, death; minus − these manifestations absent; 0 − none of the guinea pigs reacted; numbers − number of guinea pigs.

TABLE 1 (Concluded).

Sensitizing material	No. of guinea pigs		Concluding reaction to sensitizing material and number of guinea pigs				
	Controls	Desensitized	–	+	++	+++	++++
Kidney of kittens:							
7 days after infection		7	3	1	0	1	2
healthy		5	3	1	0	0	1
	3	0	3	0	0	0	0

Legend: + scratches nose with paws; ++ the same plus sneezing and shaggy fur; +++ the same plus cough, all manifestations more pronounced; ++++ excitation, jumping about, fits, death; minus—these manifestations absent; 0—none of the guinea pigs reacted; numbers – number of guinea pigs.

The analysis of corresponding experiments with saline extracts of organs of healthy mice and kittens showed that, of 30 sensitized guinea pigs, in this case 14 gave a shock reaction of intensity +, one gave a reaction of +++ and one, of ++++; in 14 guinea pigs the reaction was negative. All control animals gave a negative reaction.

It was considered to be of interest to study the analogous reaction in kittens treated for dysentery by chloramphenicol and phthalyl sulfathiazole. The animals were sacrificed on the 11th day after the beginning of treatment or on the 13th day after infection. Cultures from blood and tissue of the internal organs were negative in all cases.

Altogether, of 20 guinea pigs sensitized to extracts of the liver, spleen and kidneys of the treated kittens, 5 gave a shock reaction to the final injection of extract of the tissue of the original organ, in 4 of which the intensity was +++ and in one it was +; 15 guinea pigs gave no reaction. Of 10 guinea pigs sensitized to the organs of healthy kittens, 6 animals gave reactions of intensity ++++, +++ and +; in 4 animals there was no reaction. The control guinea pigs did not react to injection of the material.

The results of experiments* with the organs of white mice infected with streptococci and with organs of healthy animals are shown in Table 2.

Of 4 guinea pigs sensitized to "diseased" liver, all 4 reacted + to the concluding injection of extract of the same organ; in an analogous experiment with "healthy" liver, one of 4 guinea pigs reacted +. Of 6 guinea pigs sensitized to a suspension of pathological spleens, obtained from mice 5–6 hours after infection, all 6 animals reacted to the concluding injection of material from these organs, one in an intensity of ++++, 3 of +++ and 2 of ++; the corresponding experiment with the spleens of noninfected mice showed that in this case 5 of the 6 sensitized guinea pigs gave a shock reaction, one in an intensity of +++, 2 of ++ and 2 of +.

Extracts of the spleens of mice 20 hours after infection with streptococci possessed no sensitizing properties; the proteins of "healthy" organs sensitized the animals and, after desensitization with proteins from the pathological spleens, they gave a shock reaction. Tissue from kidneys of the mice, 48 hours after infection, acquired sensitizing properties.

Experiments with the organs of white mice inoculated with living tularemia and brucellosis vaccines gave negative results: under the influence of these inoculations the internal organs of the animals did not acquire sensitizing properties.

Experiments with brucellosis vaccine were carried out on 27 guinea pigs, and those with tularemia vaccine on 12.

How may the results obtained be explained? It may be assumed that the organs acquired sensitizing properties as a result of the inflammatory reaction of the animal body, leading to changes in the proteins and cells of the internal organs.

In relation to the pathogenesis of glomerulonephritis and rheumatic carditis, Cavelti [3] proved that as a result of contact between bacteria and the products of their vital activity and the tissues thereby affected, the latter acquire extraordinary properties, including those of autoimmunization.

L. A. Il'ina and R. V. Petrov [4] reported that in acute radiation sickness, terminating in death of animals, processes of protein synthesis are disturbed.

The mechanism of development of sensitizing properties in the internal organs that we studied when infected with dysentery bacilli and streptococci is not yet quite clear. There is reason to believe, however, that the processes which we studied are accompanied by the formation of autoantigens. E. D. Zhuravleva,

* Carried out in conjunction with V. P. Morozova.

TABLE 2. Results of Experiments with Organs of Mice Infected with Streptococci

Sensitizing material	No. of guinea pigs		Concluding reaction to sensitizing material and number of guinea pigs				
	Controls	Desensitized	–	+	++	+++	++++
Liver of mice:							
5 days after infection		4	0	4	0	0	0
healthy		4	3	1	0	0	0
	3	0	3	0	0	0	0
Spleen of mice:							
5-6 days after infection		6	0	0	2	3	1
healthy		6	1	2	2	1	0
	6	0	6	0	0	0	0
Spleen of mice:							
20 hours after infection		8	8	0	0	0	0
healthy		4	1	2	1	0	0
	3	0	3	0	0	0	0
Kidney of mice:							
48 hours after infection		2	0	0	1	0	1
healthy		2	0	1	0	0	1
	3	0	3	0	0	0	0

Note: Legend as in Table 1.

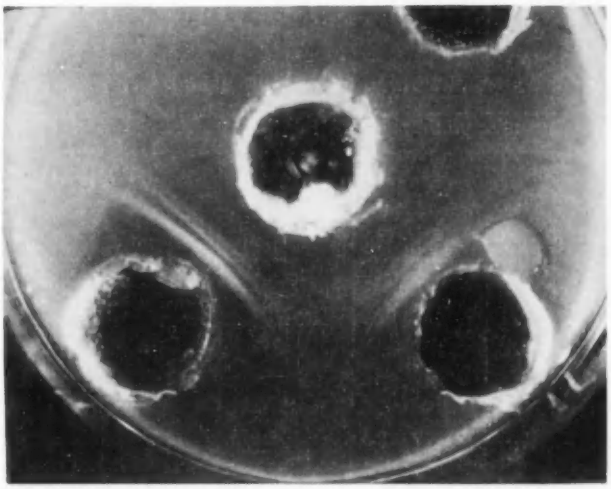


Fig. 1. Precipitation reaction in jelly with extract of the kidneys of kittens suffering from dysentery. Two foreign antigens are seen.

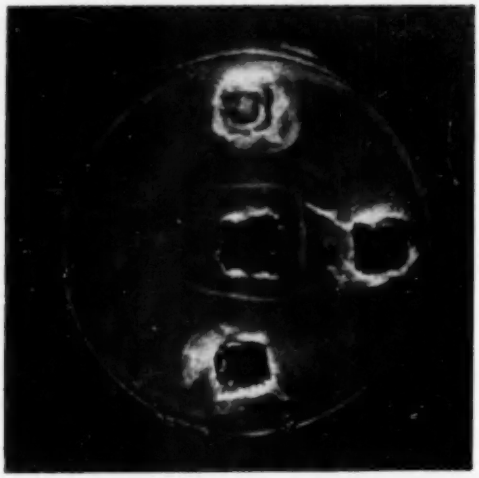


Fig. 2. Precipitation reaction in jelly with extract of the liver of kittens suffering from dysentery. One foreign antigen is seen.

for instance, using P. N. Kosyakov's technique, prepared a serum against the liver and kidney proteins of healthy kittens and of kittens suffering from dysentery. Antibodies to the causative organism of dysentery were extracted from the sera against the pathologically changed organs. The precipitation reaction in jelly with antisera against "healthy" kidney gave one line of precipitate, and against "healthy" liver two, demonstrating the presence in these organs of one or two (liver) antigens. The analogous reaction with serum against "dysenteric" liver and kidneys and with their extracts gave three lines of precipitate (Figs. 1 and 2).

By means of the precipitation reaction in jelly, the presence of two foreign antigens in pathological kidneys and of one in liver was thus demonstrated.

SUMMARY

The parenchymatous organs of kittens and white mice aquire specific sensitizing properties during the process of experimental infection of these animals with the causative agents of dysentery and streptococci. Vaccination of white mice against tularemia and brucellosis is not associated with the above phenomena.

LITERATURE CITED

1. K. V. Bunin, in: The Role of Immunity and Allergy in the Treatment of Infectious Diseases [in Russian] (Moscow, 1958).
2. M. V. Zemskov, Byull. Eksp. Biol. Med. 2, 66 (1957).†
3. L. A. Zil'ber, in: The Basis of Immunology [in Russian] (Moscow, 1958).
4. L. A. Il'ina and R. V. Petrov, Tsitologiya 1, 3, 289 and 292 (1958).
5. P. N. Kosyakov, in: Antigenic Substances of the Body and Their Importance in Biology and Medicine [in Russian] (Moscow, 1954).
6. R. V. Petrov and L. I. Il'ina, Theses of the First All-Union Conference on Radiology [in Russian] (1956).

†Original Russian pagination. See C. B. translation.

ON THE IMMUNOLOGIC CHARACTERISTICS OF SERUM PROTEINS

Part 1. Induction of Anaphylaxis by Isolated Fractions of Blood Serum Proteins

Yu. S. Tatarinov

Department of Biological Chemistry (Acting Head – Docent Yu. S. Tatarinov)
A. V. Lunacharskii Astrakhan Medical Institute (Director-Candidate Med. Sci. I. L. Alamdarov)
(Presented by Active Member AMN SSSR V. V. Parin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 97-100, October, 1960
Original article submitted January 18, 1960

At present, one may consider as established that individual electrophoretically pure fractions of serum proteins are immunologically heterogeneous [2, 5, 7, 8, 9].

Data are also available dealing with the possibility of specific cross reactions between albumins and globulins [3].

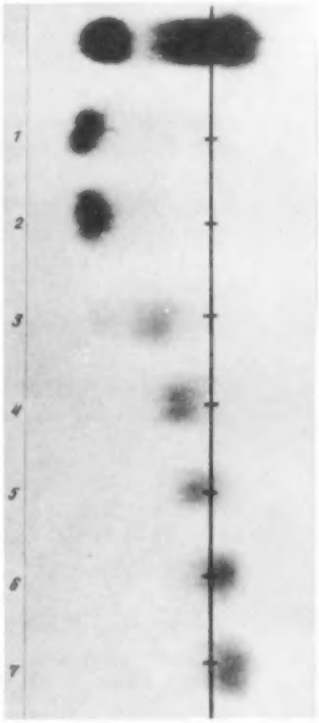


Figure. Electrophoresis of isolated protein fractions separated on starch block (paper B 12 x 40 cm, 450 v, 2.5 ma, 4 hr). 1,2) Albumins; 3) α_1-; 4) α_1-; 5) β-; 6) γ_1-; 7) γ_2-globulins; above - normal horse serum.

In this connection, it appeared interesting to clarify whether it is possible to differentiate anaphylactogenic properties of different isolated fractions of serum proteins.

The available pertinent data from literature are contradictory. Experimental results reported by Svingendow and his co-workers [4] were not substantiated by experiments of N. A. Gorbunova and T. M. Tsipin [1]. The former authors believe that it is not essential to introduce different fractions of serum proteins (albumins and globulins) as sensitizing and challenging injections for induction of anaphylactic shock in guinea pigs.

To resolve this question we utilized, as a model of anaphylactic reaction, anaphylactic shock in guinea pigs.

METHODS

Fractionation of serum. Separation of normal horse serum proteins was carried out in an apparatus similar to that described by Kunkel, utilizing a starch block (1x13 x26 cm) in median-veronal buffer (pH 8.6, ionic strength 0.1) for 48 hr under 17.3 v/ cm potential gradient and 10 ma current. Visual observation of migration of albumins and globulins in the course of electrophoresis has been insured by addition of a small quantity of Evans' blue (T-1824) to the serum.

Isolation of protein fractions. A part of the starch block containing proteins was cut into individual cross sections 1 cm wide. Proteins were extracted from starch with the help of the median-veronal buffer. The amount of protein in each sample was determined using Lowry's method [6] and the distribution of proteins in the starch block was plotted graphically. Control of purity of protein fractions isolated from starch was carried out with the help of micropaper electrophoresis. The protein solutions obtained wereanalyzed in cold against physiological saline for 30 hr.

Sensitization and shock. Experiments were carried out using male and female guinea pigs weighing 200 to 450 g. One or two sensitizing injections were given sub-

Induction of Active Anaphylaxis in Guinea Pigs by Purified Fractions of Serum Proteins

Animal number	Wt. of animal (in g)	Sensitizing injection			Shocking injection				Nature of anaphylactic reaction
		date (1959)	protein fraction	amount of protein (in mg)	day of reinoculation of antigen	protein fraction	amount of protein (in mg)	volume of solution (in ml)	
73	435	Jan. 10, 12	A	1.5	20	A	3.5	1.0	++++
74	230	Jan. 10, 12	A	1.5	20	α_1-g	3.5	1.2	+
					21	A	3.5	0.1	++++
70	324	Jan. 10, 12	A	1.5	20	α_2-g	3.0	1.1	—
					21	A	3.5	0.1	++++
125	240	Nov. 3	A	1.0	21	γ-g	4.0	1.0	—
					21	A	3.2	0.1	++++
119	290	Nov. 3	α_1-g	1.0	21	A	3.5	0.4	++++
120	360	Nov. 3	α_2-g	1.0	21	A	3.5	0.4	++++
91	350	June 16	α_2-g	1.0	21	α_1-g	2.5	1.0	++++
90	350	June 16	α_2-g	1.0	21	β-g	1.2	0.8	++++
92	440	June 16	α_2-g	1.0	21	γ-g	1.5	0.5	++++
93	415	June 16	β-g	1.0	21	A	7.0	1.0	—
122	410	Nov. 3	β-g	1.0	21	A	4.0	0.5	—
					21	γ-g	4.0	1.0	++++
126	225	Dec. 4	γ_1-g	2.0	22	A	6.8	0.9	—
					22	α_1-g	2.0	1.0	++++
128	305	Dec. 4	γ_2-g	1.5	22	A	5.2	0.7	—
					22	α_2-g	2.8	0.9	+++
99	420	June 16	γ_2-g	1.0	21	β-g	2.0	1.0	++++
130	285	Dec. 4		0.5	22	γ_1-g	2.7	0.9	++++

Legend: A—albumins; g—globulins; ++++ severe anaphylactic shock leading to death in 2-5 minutes; +++ prolonged anaphylactic shock leading to death a few hours after injection of antigen; + anaphylactic reaction in the form of scratching, coughing, shaking, and with rapid passing of these symptoms; — without symptoms characteristic of anaphylactic shock.

cutaneously using a solution of electrophoretically pure protein fraction (from 0.2 to 5 mg protein).

Shocking dose (1 to 7 mg protein) was administered intracardially on the 20-22nd day after the sensitizing dose. Forty-five guinea pigs were used in these experiments.

RESULTS

As can be seen from the table, all the protein fractions of normal horse serum were equally anaphylactogenic when tested on guinea pigs, and a pronounced anaphylactic shock was observed following administration of shocking dose of the corresponding antigen. The results obtained confirm the known position about the specificity of anaphylactic phenomena being within the boundry of one and the same type of serum proteins. However, using albumins for sensitizing dose and globulin (except α_1- globulins) for shocking dose makes it impossible to bring about a general anaphylactic reaction. Anaphylactic shock was also absent when β-and γ- globulins were used for sensitization and albumins for shocking dose. Cross anaphylactic reactions occasionally observed when α- globulins were used for sensitization and albumins for shocking dose are apparently conditioned in that these globulin fractions contain different amounts of albumin impurities. As can be seen on the figure, α_1- globulins isolated from a starch block have noticeable amounts of proteins with electrophoretic mobility of albumins. However, it is not possible to uncover proteins with electrophoretic mobility of albumins in paper electrophoresis of α_2- globulins. However, if we consider that active sensitization is possible by introduction of 0.001 mg of protein, then the cross anaphylactic reactions may be also carried out because of insufficient homogeneity of α_2-globulins, although this is not made apparent upon paper electrophoresis.

When the isolated globulin fractions are used for sensitization, anaphylaxis can be brought about by using any globulin fraction as shocking dose. In addition specific desensitization may be accomplished using any of these fractions. However, guinea pigs sensitized with globulins, and which were shocked by injections of albumins, remain sensitive to subsequent injection of globulins. These data point to great similarity in anaphylactogenic properties of isolated fractions of serum globulins separated by means of starch block electrophoresis.

Results obtained by us are indicative of anaphylactogenic specificity of electrophoretically pure fractions of albumins in comparison with β-and γ-globulins.

SUMMARY

Preparatory isolation of electrophoretically pure protein fractions from normal horse serum was carried out with the help of apparatus of Kunkel type in a starch block (1x13x26 cm) with 450 v, 10 ma during 48 hours.

Experiments on guinea pigs with production of active anaphylaxis have shown that albumins and globulins possess different anaphylactogenic specificity, while individual fractions of globulins possess identical immunogenic properties and are capable of giving cross anaphylactic reactions.

LITERATURE CITED

1. N. A. Gorbunova and T. M. Tsipin, Byull. Éksp. Biol. Med. 36, 10. 46 (1954).
2. S. Ya. Kaplanskii, A. E. Gurvich and L. K. Starosel'tseva, Biokhimiya 23, 1, 114 (1958).*
3. Yu. A. Khavkin, in: Scientific Works of Students of Tashkent Medical Institute [in Russian](1956) p. 23.
4. Svingendow, et al., as cited by N. A. Gorbunova and T. M. Tsipin.
5. G. Berg, F. Scheiffarth, H. Gotz, et al., Klin. Wschr. 37, 144 (1959).
6. O. H. Lowry, N. J. Rosebrough, A. L. Farr, et al., J. Biol. Chem. 193, 265. (1951).
7. J. Oudin, Ann. Inst. Pasteur 85, 336 (1953).
8. R. J. Slater, Arch. Biochem. a. Biophys. 59, 33 (1955).
9. C. A. Williams and P. Grabar, J. Immunol. 74, 404 (1955).

* Original Russian pagination. See C. B. translation.

THE EFFECT OF ANTIRONIDASE* SERUM OF GOATS ON THE METASTASIZATION OF BROWN-PEARCE CARCINOMA IN RABBITS

I. N. Maiskii and N. A. Kozlova

Laboratory of Noninfectious Immunology (Head-Prof. I. N. Maiskii) of the Institute of Experimental Biology (Dir.-Prof. I. N. Maiskii) of the AMN SSSR, Moscow
(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 101-105, October, 1960
Original article submitted May 20, 1960

According to the investigations of a number of authors [1, 3, 5, 7, 9, 13], malignant tumors and their adjacent tissues [3, 7] contain more than the normal quantity of spreading factor of the type of the enzyme hyaluronidase. The most intensive hyaluronidase activity is shown by metastases of malignant neoplasms [5, 6]. The addition of hyaluronidase to inoculated tumor material [2, 10], the injection of this enzyme into the tumor or near it [4, 14], and also the action of the enzyme on the body as a whole [8, 12] promotes the more intensive growth of the primary tumor and stimulates metastasis formation.

The necessity thus arises for the search for methods of inactivation of the spreading factor of malignant tumors. One such method may be the use of "antifactor" sera. Very little work, however, has been done on the study of this problem. For instance, it has been shown by experiments in vitro [2] that the activity of the hyaluronidase of Ehrlich's adenocarcinoma is depressed under the influence of an antitumor serum.

It has been reported [11] that the incidence of recurrence in mice, after extirpation of spontaneous tumors of the mammary glands, is decreased by administration of antihyaluronidase serum of rabbits.

The object of the present research was to study the action of the antihyaluronidase sera of goats on the process of metastasization of a Brown-Pearce carcinoma in rabbits.

EXPERIMENTAL METHOD AND RESULTS

The antihyaluronidase sera which we used in the experiments were obtained by the intravenous immunization of goats with a saline extract of rabbit's testis and of rodinase, and also a serum against the Brown-Pearce tumor, possessing antihyaluronidase activity. The sera were obtained on the 9-14th day after the last immunizing injection of extract.

The sera used in the experiments possessed the following titer: 1) the antironidase serum reacted with the enzyme from the rabbit's testis in a dilution of 1:128 and with rodinase in a titer of 1:512; 2) the serum of the goat, immunized with extract of rabbit's testicular tissue, depressed the action of the homologous enzyme in a dilution of 1:512 and rodinase in a dilution of 1:64; 3) the titer of antibodies against the hyaluronidase of rabbit's testes in the serum of the goat, immunized with extract from tissue of a Brown-Pearce tumor, was 1:256, and against rodinase the titer was 1:16.

The experiments were conducted on 20 male chinchilla rabbits. Simultaneous intratesticular injections of 1 ml of a 25% suspension of cells from a Brown-Pearce tumor and from metastases in the liver and omentum were given to all the rabbits.

On the eighth day after inoculation of the tumor, the testes with the developing tumors were removed. After operation, the rabbits were divided in accordance with their weight into five equal groups with four rabbits in each.

On the day after removal of the tumor, i. e., on the ninth day after inoculation of the carcinoma, the rabbits were injected with sera. The rabbits of the first group received the serum of a goat immunized with ronidase. The animals of the second group received injections of the serum of a goat against extract of rabbit's testicular tissue. The third group of rabbits were injected with the serum of a goat immunized with Brown-Pearce tumor tissue. The fourth group were injected with normal goat serum. The animals of the fifth group received no serum.

The first injection of 6 ml of serum was made into the marginal vein of the ear. The next three injections of serum, in a dose of 5 ml each, were given into the thigh muscle. The interval between the first and sec-

* Ronidase is a preparation with hyaluronidase action, from the testis of the ox, marketed by the Mikoyan Meat Combine, Moscow.

TABLE 1. Degree of Metastasization of a Brown-Pearce Carcinoma in Rabbits after Removal of the Primary Focus of the Tumor and Injection of Sera (21st Day of Transplantation of Tumor)

Group	Serum	Rabbit no.	No. of organs affected	Number of metastases[1] present in one affected organ	Omentum		Spermatic cord	
					degree of involvement	wt. (in g)	deg. of involvement	wt. (in g)
First	Against rohidase	2 973	—	—	—	5.0	—	1.5
		3 674	3	1	+	10.0	+	2.0
		2 190	5	7	+	8.5	+++	15.0
		2 402	4	15	+	12.0	+	5.0
	Mean		3,0	5,8		8.9		5.9
Second	Against rabbit's testes	14	2	16	+	7.5	—	1.5
		67	2	8	—	8.5	—	2.0
		3 741	11	270	+++	39.0	+++	15.0
		936	Died from accidental causes					
	Mean		5.0	98,0		18.3		6.2
Third	Against Brown-Pearce tumor	1 311	1	3	—	15	—	1.5
		1 028	10	32	+++	100	+++	26
		12	7	72	+++	35.5	+++	17
		2 939	9	141	++	20	+++	18
	Mean		6,8	62,2		42,6		15,6
Fourth	Normal goat	23	7	19	+	21	+++	18
		185	8	101	+++	41	++	11
		54	9	126	++	20	++	8
		3 065	11	653	+	7,5	+	6
	Mean		8,8	224,8		22,4		10,8
Fifth	No serum injected	1 369	8	13	++	33	++	9
		18	9	49	+	16	+	5
		46	6	127	++	26	+	8
		20	10	547	+++	82	+++	22
	Mean		8,3	184		39,3		11

Conventional signs: – metastases absent; + solitary metastases (from 1 to 10); ++ multiple metastases (from 11 to 50); +++ innumerable metastases (over 50).

[1]Metastases in the spermatic cord and omentum were disregarded when the number of metastases present in an affected organ was being counted.

ond injections was 2 days, and between the others, 3 days. In order to avoid anaphylactic shock, this volume of serum in all cases was injected as fractionated doses. All the animals were sacrificed on the 21st day after inoculation of the tumor.

The effectiveness of action of the sera was assessed by the extent to which the organs were affected by metastases, taking into account the number of affected organs and the number of metastases in each.

The mestastases were counted as follows. In organs containing a smaller number of metastases, those on the surface were counted first, and later those in serial sections of the organs approximately 5 mm thick. The method of counting in organs containing a large number of metastases was rather more complicated. In these cases the number of metastases per unit weight of the organ was estimated, and then the total number for the whole organ was calculated. In organs in which it was not always possible to count the number of metastases, because the organ consisted of a conglomeration of confluent nodes (testis, omentum, etc.) the degree of involvement was designated conventionally

TABLE 2. Degree of Metastasization of a Brown-Pearce Carcinoma in Rabbits after Removal of the Primary Focus of the Tumor and Injection of Sera (37th Day of Transplantation of Tumor)

Group	Serum	Rabbit no.	Number of organs affected	Number of metastases[1] present in one affected organ	Omentum		Spermatic cord	
					degree of involvement	wt. (in g)	degree of involvement	wt. (in g)
First	Against ronidase	4	—	—	—	7	—	1.5
		55	—	—	—	18	—	1.5
		64	3	2	—	7	—	1.2
		42	5	3	+	7	++	12
		33	6	105	+	12	+	1.6
		38	8	136	+	14	++	14
	Mean . . .		3.7	41.0		10.8		5.8
Second	Against Brown-Pearce tumor	37	7	2	+	7	+	7
		24	9	61	+	5	+	1.6
		36	11	141	+++	27	++	6
		62	8	176	+++	107	—	1.5
		78	8	275	++	8	++	9
		3	11	200	+++	117	+++	53
	Mean . . .		9.0	142.5		45.1		13.0
Third	Normal goat	40	10	63	++	24	+++	20
		70	8	86	+++	53	+++	49
		35	11	446	+++	64	+++	53
		26a	11	629	++	8	+++	35
	Mean . . .		10	306		37.2		39.2

Note: Legend as in Table 1.

by plus signs, and the degree of involvement was further compared in these organs by the gravimetric data.

The experimental results are shown in Table 1. The average number of organs affected by metastases, and the average number of metastases present in one affected rabbit's organ were least in the group of animals receiving antironidase goat serum.

Of each 12 organs examined, on the average three were affected, and for each affected organ in the rabbits of this group there were 5.8 metastases.

The injection of antitesticular and antitumor sera was almost equally effective, slightly lowering the incidence of affected organs (5 affected organs in the second group and 6.8 in the third) and the number of metastases in the organ (98 metastases in the second group and 62 in the third) by comparison with the control animals receiving nonimmune goat serum or no serum whatsoever (the respective values were 8.8 and 8.3 affected organs and 224.8 and 184 metastases in an affected organ).

When the gravimetric data were compared, the smallest values were also observed in the rabbits of the first group, which received injections of antironidase serum [weight of omentum 8.9 g, mean weight of spermatic cord 5.9 g in the first group, compared with a mean weight of 22.4 g for the control groups (4 and 5), that of the omentum being 39.3 g and of the spermatic cord 10.8-11.0 g].

By comparison with the antironidase serum, the serum of the goat immunized against rabbit's testicles had almost the same effect on the development of the tumor in the spermatic cord of the second group of rabbits (weight 6.2 g).

The remaining sera had no significant effect either on the metastasization of the carcinoma in the omentum or on the development of the tumor in the spermatic cord.

The results obtained show that as a result of the action of antihyaluronidase immune sera on metastasization of a Brown-Pearce tumor in rabbits, the greatest depression of metastasization of the carcinoma was produced by the serum of the goat immunized with ronidase. In order to verify these results, in a second series of experiments 16 rabbits were used, divided into 3 groups

(6 rabbits in the first and second groups and 4 in the third group). The animals received injections as follows: first group – the serum of a goat immunized with ronidase; second group – a serum against Brown-Pearce tumor; third group – the serum of a normal, unimmunized goat.

For inoculation we used a 40% tumor suspension (0.5 ml). The testis with the tumor was removed on the seventh day after inoculation, and injection of the serum began on the eighth day, the same method being used as in the first experiment. The rabbits were kept under observation until the 37th day of development of the tumor, when those animals still alive were sacrificed. The effect of each serum was judged by the same criteria as in the first series of experiments (number of organs affected by metastases, number of metastases in an organ, and gravimetric data).

In this series of experiments too, the results obtained confirmed those of the preceding series. The least values of the degree of metastasization were observed in the first group of rabbits, receiving antironidase serum. In the experimental rabbits an average of from 3 to 7 organs was affected, compared with 10 in the controls (third group); an affected organ in the first group contained 41 metastases, compared with 306 in the third group. The same pattern was found for the organs for which the gravimetric data served as criteria of degree of involvement (weight of omentum in experimental series 10.8 g, control series 37.2 g; weight of spermatic cord in experimental series 5.8 g, control series 39.2 g). Some depression of the degree of metastasization was observed after injection of the antitumor serum (second group), as shown by a decrease in the number of metastases present in one affected organ and by a decrease in the weight of the spermatic cord by comparison with that in the control group (third group).

It must also be pointed out that among the animals of the first group no deaths had taken place on the 37th day, whereas in the control group, receiving normal goat serum, all the animals had died at this time (mean duration of survival of the rabbits of the control group was 28 days). Two rabbits of the group receiving antitumor serum were alive on the 37th day.

Thus the results of the second series of experiments also demonstrate a decrease in the degree of malignancy of the tumor in the rabbits as a result of the injection of the serum of goats immunized with ronidase. We consider that the inhibiting action of antironidase sera on the metastasization of the carcinoma is due to the depression of hyaluronidase by the antibodies.

SUMMARY

It may be concluded from these experiments that the administration of an antironidase serum to rabbits after the preliminary removal of a primary Brown-Pearce tumor has an inhibiting effect on metastasization of the carcinoma. In our opinion this confirms the role of hyaluronidase in the process of metastasization of the tumor.

LITERATURE CITED

1. K. P. Balits'kii, Med. Zhur. Ukrain. 19, 2, 31 (1949).
2. K. P. Balits'kii, Med. Zhur. Ukrain. 20, 2, 53 (1950).
3. I. N. Maiskii and N. A. Troitskaya, Byull. Éksp. Biol. Med. 32, 12, 464 (1951).
4. M. D. Podil'chak, Med. Zhur. Ukrain. 21, 5, 52 (1951).
5. M. D. Podil'chak, Vrachebnoe Delo, 1, 33 (1952).
6. M. D. Podil'chak, Abstracts of Proceedings of the First Ukrainian Conference of Pathophysiologists [in Russian] (Kiev, 1959) p. 170.
7. N. A. Troitskaya, in: Problems of the Immunology of Normal and Malignant Tissues [in Russian] (Moscow, 1956) p. 105.
8. V. P. Shekhonin, in: Reactivity of Cancer Patients, Doctorate dissertation [in Russian] (Moscow, 1952) Vols. 1 and 2.
9. F. S. Duran-Reynals and F. W. Stewart, Am. J. Cancer 15, 2790 (1931).
10. M. A. Kraul, Naturwissenschaften, 42, 587 (1955).
11. A. Lacassagne, J. Loiseleur and G. Rudali; Compt. rend. Acad. Sc. 244, 1587 (1957).
12. W. Lührs and H. Willig, Arch. Geschwulstforsch 6, 183 (1954).
13. M. McCutcheon and D. R. Coman, Cancer Res. 7, (1947).
14. W. L. Simpson and A. R. Ayengar, Anat. Rec. 97, 369 (1947).

A 5-HYDROXYTRYPTAMINE FROM Hippophae rhamnoides AS AN ANTITUMOR PREPARATION IN EXPERIMENTS ON ANIMALS

E. Ch. Pukhal'skaya, M. F. Petrova, and G. P. Men'shikov

Laboratory of Experimental Chemotherapy (Head-Corresponding Member AMN SSSR Prof. L. F. Larionov) and the Laboratory of the Chemistry of Natural Substances (Head-Prof. G. P. Men'shikov) of the Institute of Experimental and Clinical Oncology (Dir.-Active Member AMN SSSR N. N. Blokhin) of the AMN SSSR, Moscow

(Presented by Active Member AMN SSSR N. N. Blokhin)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 105-110, October, 1960
Original article submitted February 11, 1960

Two years ago the first report was published of the positive results of a preliminary trial of an extract from the bark of the sea buckthorn (Hippophae rhamnoides, fam. Elaeagnaceae) for its antitumor activity [3]. Later work showed considerable inhibition of the growth of various tumors of animals by the action of this extract [4]. In May, 1958,* G. P. Men'shikov and M. F. Petrova isolated from the extract a group of bases, which was conventionally named preparation Hr, and which contained the active principle [2].

In 1959 the same workers succeeded in isolating one of the bases of this group with Rf-0.4 in crystalline form (as the hydrochloride) and identified it as 5-hydroxytryptamine. As we know, 5-hydroxytryptamine is also present in the body of man and animals as one of the physiologically active biogenic amines, and is called serotonin or enteramine [6-8]; this is the first time that it has been isolated from plants (M.F. Petrova and G. P. Men'shikov, 1960). A report of the method of its isolation will be given by the authors elsewhere.

In the present communication the results are described of experiments with 5-hydroxytryptamine hydrochloride from sea buckthorn, as a preparation which we have called "hippophain," in rats and mice with transplanted tumors.

EXPERIMENTAL METHOD

Hippophain is readily soluble in water. The solution has a weakly acid reaction and is readily absorbed after subcutaneous injection without causing irritation of the surrounding tissues. Since the solution darkens on heating, we prepared it in aseptic conditions without sterilization. Its toxic action was investigated in rats, which showed much greater sensitivity towards it than did mice. All the therapeutic experiments were carried out on equal numbers of animals (mice and rats): ten in the experimental group and ten in the control. For the treatment of the tumors we used only those doses which did not cause death when given by subcutaneous injection to animals of that particular species for a period of 14 days. The tumors were inoculated subcutaneously, with the exception of Ehrlich's tumor, which was inoculated intramuscularly. Treatment of the animals was started on the 5-7th day after inoculation of the tumor [except in experiment No. 8 (Table 1), which was started on the ninth day] and continued for 12-15 days. In these experiments the preparation was injected subcutaneously every day. The inhibiting action on the tumors was estimated by the difference between the average weight of the tumors in the control and experimental series, expressed as a percentage of the control weight.

EXPERIMENTAL RESULTS

The pharmacological action of serotonin has been described by a number of authors [5-8]. Since its principal toxic effect is known to be its action on the vascular system and the smooth muscle of the intestine, and also on the kidneys, we were particularly interested in the reactions of these organs to the administration of a therapeutic dose, i. e., of a dose causing significant inhibition of growth of the tumor (see Table 1), and also in the upper limit of this dose. The subcutaneous or intraperitoneal injection of 10-20 mg/kg of hippophain in physiological saline into rats causes depression, restriction of mobility, changes in the rhythm and the strength of respiratory movements and an increase in the peristalis of the intestine with a transient relaxing

* The presence of unidentified alkaloids in the sea buckthorn was first mentioned by P. S. Massagetov [1].

TABLE 1. The Action of Hippophain on Tumors

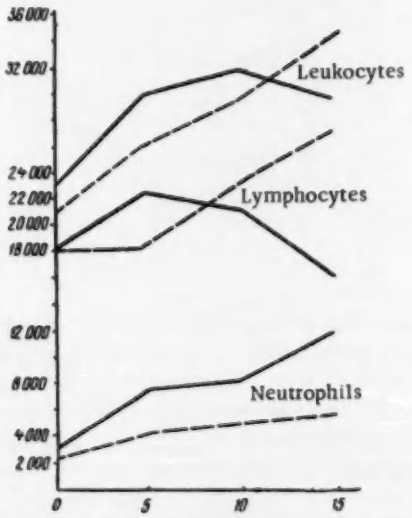
Expt. No.	Tumor	Dose (in mg/kg)	Mean wt. of tumor at end of expt. (in g)		Inhibition of tumor growth (in %)	P (by Student's method
			expt.	control		
	Mice					
1	Ehrlich's tumor (inoculated intramuscularly)	3	4.2	7.1	42	<0.0001
2	The same	5	3.6	7.1	49	<0.0001
3	" "	10	3.3	5.7	42	<0.0001
4	" "	20	3.0	5.7	47	<0.0001
5	" "	50	3.0	5.7	49	<0.0001
6	" "	100	3.4	6.7	49	=0.001
7	" "	200	2.4	7.0	62	<0.0001
8	" " (experiment begun on large tumors weighing 2 g)	20	2.6	4.9	47	=0.003
9	Hardening-Passey melanoma	10	3.5	6.3	44	=0.2
10	The same	20	3.8	6.8	44	=0.02
11	" "	5	3.2	6.8	53	<0.0001
12	Crocker's sarcoma	50	1.2	6.2	81	<0.0001
13	Sarcoma 37	50	1.8	4.1	56	=0.003
14	Carcinoma OZh-5 of the forestomach of the mouse	20	4.9	4.0	0	
15	Lymphosarcoma LIO-1	200	2.7	2.7	0	
	Rats					
16	Sarcoma 45	5	16.5	15.0	0	
17	The same	10	14.0	15.0	0	
18	" "	20	5.6	34.8	84	<0.0001
19	Jensen's sarcoma	10	0.9	30.6	97	<0.0001
20	Geurin's carcinoma	5	6.3	17.2	60	=0.058
21	The same	10	3.4	17.2	80	=0.008

action. On intraperitoneal injection, all these phenomena occur at once, but on subcutaneous injection they arise after 10-15 min. After intraperitoneal injection of 50 mg/kg, death of the animal occasionally ensues. An increase of the dose to 300-500 mg/kg, injected intraperitoneally, causes ataxia and, in certain rats, a severe spasm of the vessels of the optic fundus ("white eyes"); these phenomena all pass off after 4-5 hr. With these doses, death also supervenes occasionally in animals most highly sensitive to the action of the preparation. They die within 24 hr after the injection, showing signs of disturbance and gradual weakening of respiration and of cardiac activity. At necropsy the dilated, flabby heart and the parenchymatous organs of the abdominal cavity are overfilled with venous blood. We did not establish with accuracy the value of LD_{50} for rats, but it is considerably more than 500 mg/kg. After administration of therapeutic doses of 5 and 10 mg/kg for 14 days to mice and rats we observed no dystrophic changes whatsoever in the internal organs. A dose of 20 mg/kg at the end of the experiment caused in individual rats the development of necrotic areas in the kidneys. This also took place as a result of the administration of a dose of 10 mg/kg to rats for a period of 30 days. Attention must be drawn to the marked individual sensitivity to the toxic action of the preparation. One of the authors tried out a single injection of the preparation on himself (40 mg as a 4%

solution intramuscularly). Ten minutes after the injection a reaction of the cardiovascular system developed, in the form of considerable reddening of the skin of the face and neck,, a feeling of heaviness in the head and, in particular, in the region of the heart, All these phenomena passed off after 15 min. The arterial pressure and the pulse rate were not altered during this reaction.

The action of hippophain on hemopoiesis was studied in rats in which a sarcoma 45 had been inoculated subcutaneously, in a therapeutic experiment lasting 2 weeks on 10 animals, at the end of which growth of the tumors was retarded under the action of the preparation to the extent of 84% of the control values (see Table 1, experiment no. 17). The controls used were ten rats with tumors but not receiving treatment. The preparation was injected subcutaneously in a dose of 20 mg/kg daily. The blood was examined once before treatment and three times in the course of treatment, every 5 days. From determinations of the number of leukocytes (in a counting chamber) and of the leukocyte formula (from blood films), the number of lymphocytes and neutrophils in 1 mm^3 of blood was determined separately, after which the arithmetic mean values of these indices for all the rats were calculated, and these were plotted on a graph. The total leukocyte count in the control animals (see top graph in figure, broken line) rose continuously in accordance with the growth of the tumor and with the increasing destruction of its central part. In the experimental series (continuous line), in spite of the considerable inhibition of the tumor growth by the action of the preparation, a moderate leukocytosis was also found, and this diminished slightly on the 15th day. As is shown by the middle graph (see figure, lymphocytes), this decrease results from a slight fall in the number of lymphocytes (by 15% below the initial level) in the blood of the treated animals. On the other hand, however, the preparation has a stimulating action on the segmented neutrophils: their number rose considerably, with no shift to the left, and at the end of the experiment was 70% above the original level (see figure, neutrophils). This is seen particularly demonstratively in the rats, in whose blood there are far more lymphocytes than neutrophils. Under the action of the preparation, at the end of the experiment their blood closely resembled human blood in the proportions of these cells. No other abnormalities were observed during the examination of the films and calculation of the leukocyte formulae. No action of the preparation on the number and external appearance of the red cells could be found. The injection of the same dose per kg body weight into mongrel mice (without tumors) for the same period of time gave rise to no changes in the total leukocyte count and in their relative proportions.

The action of hippophain on tumors. The antitumor action of the preparation is distinguished by a wide spectrum, for as a result of its action on 9 strains of transplanted tumors, 7 were found to show some degree of inhibition of growth (see Table 1). No inhibition whatsoever of growth of a squamous cell carcinoma OZh-5 of the forestomach of the mouse (line C_3HA) and of a lymphosarcoma LIO-1 (in a mouse of line Afb) took place. However, those strains of tumor which were found to be sensitive to the action of this preparation varied very considerably in the character of their sensitivity to it. This is clear from a comparison of the inhibiting action of different doses on growth of the Ehrlich's tumor and of the sarcoma 45 (see Table 1). Inhibition of growth of the Ehrlich's tumor, caused after treatment with a daily dose of 5 mg/kg for 12 days, amounted to about 50%. Subsequent experiments in which the daily dose was increased to 200 (!) mg/kg gave no significant increase in the strength of the inhibiting action. In contrast to this, sarcoma 45 gave no inhibition of growth when injected in doses of 5 and 10 mg/kg; inhibition developed only after a dose of 20 mg/kg. The other rat tumors – Jensen's sarcoma and Guerin's carcinoma – were more sensitive to this preparation, for they reacted in varying degree to doses of 5 mg/kg (Guerin's carcinoma) and 10 mg/kg (Jensen's sarcoma). The same applies to the melanoma of mice (Harding-Passey strain). Only the action of a large dose has so far been tested on other tumors. Administration of the preparation by mouth had no antitumor effect.



The action of hippophain (20 mg/kg) on the blood of rats with sarcoma 45. Along the axis of ordinates – mean number of leukocytes in 1 mm^3 blood. Along the axis of abscissas – time in days from the beginning of treatment. – – – control; —— experiment.

TABLE 2. Comparison of the Antitumor Action of Two Therapeutic Forms of Serotonin: 5-Hydroxytryptamine Hydrochloride (Hippophain) and 5-Hydroxytryptamine-Creatinine Sulfate (5-HTCS)

Expt. No.	Preparation	Dose (in mg/kg)	Tumor	Commence day of expt. (after transplantation)	Result: weight of tumor (in g) expt.	Result: weight of tumor (in g) control	Result: inhibition (in %)
1	Hippophain	5	Ehrlich's tumor, intramuscularly	5-th	3.4	5.9	42
	5HTCS[1]	10	The same	5-th	4.5		24
2	Hippophain	5	Jensen's sarcoma, subcutaneously	4-th	14.2		67
	"	10	The same	4-th	5.8	43.0	87
	5HTCS[2]	20	" "	4-th	13.4		71

[1]Synthesized by Prof. N. N. Suvorov (All-Union Chemo-Pharmaceutical Research Institute, Moscow)

[2]Synthesized by M. F. Petrova (Institute of Experimental and Clinical Oncology of the AMN SSSR, Moscow)

Since synthetic 5-hydroxytryptamine (serotonin), which is widely used in laboratory practice, is usually obtained as a complex with creatinine sulfate, we carried out experiments to compare the action of the hydrochloride of 5-hydroxytryptamine (hippophain) and of synthetic 5-hydroxytryptamine-creatinine sulfate on tumors. Adequate doses were computed according to the content of the pure base: the hydrochloride contains approximately $\frac{2}{3}$ of the pure base, whereas the complex with creatinine sulfate contains approximately $\frac{1}{3}$; we therefore gave twice the dose of the complex with creatinine sulfate.

The results showed (Table 2) that 5-hydroxytryptamine, in the form of a complex with creatinine sulfate, has a much weaker action on tumors than the hydrochloride. For instance, in an experiment on Jensen's sarcoma, 5-hydroxytryptamine-creatinine sulfate in a dose of 20 mg/kg caused almost the same degree of inhibition as that caused by hippophain in a dose of 5 mg/kg, i. e., the inhibiting activity of the former is approximately half† as strong as that of the latter.

There are reports in the literature of investigations into the action of synthetic serotonin (in the form of a complex with creatinine sulfate) on tumors in animals [9, 10]. The authors of these reports, however, obtained no antitumor effect with serotonin, but came to the opposite conclusion, namely that it has a stimulating action on the growth of experimental tumors. The discrepancy between these authors' findings and our own may, possibly, be explained by differences in the form of the preparation (see Table 2) or in the method of its administration, or, finally, by the use of different tumor strains for the investigation.

It follows from these results that hippophain may be of interest for trial in clinical oncological practice.

SUMMARY

5-hydroxytryptamine was recently isolated from the bark of Hippophae rhamnoides by G. P. Men'shikov and M. F. Petrova. The results of investigation of the action of the hydrochloride of the alkaloid upon the organism and tumors of mice and rats are presented. The preparation was named hippophain. The antitumor effect of hippophain is characterized by a wide range of action, the preparation inhibiting the growth of 7 out of 9 animal tumors (various types). However, the tumors do not resolve completely. Hippophain exercised no antitumor effect when administered per os.

The side effects of the preparation are connected with action upon the cardiovascular and respiratory systems.

The effects of a large dose of the preparation (20 mg/kg) upon hemopoiesis was studied during a 2-week treatment of sarcoma 45.

A moderate leukocytosis in the peripheral blood was observed under the action of hippophain. The absolute number of neutrophils per 1 mm^3 of the blood increases by 70% in comparison with the initial level (without any shift to the left) whereas the number of lymphocytes is slightly reduced.

LITERATURE CITED

1. P. S. Massagetov, Zhur. Obshch. Khimii 16, 1, 139 (1946).
2. M. F. Petrova, E. Ch. Pukhal'skaya and G. P. Men'shikov, Byull. Éksp. Biol. Med. 47, 2, 102 (1959).‡
3. E. Ch. Pukhal'skaya, M. F. Petrova and P. S. Massagetov, Byull. Éksp. Biol. Med. 43, 6, 57 (1957).‡

† As in original–Publisher's note.

‡ Original Russian pagination. See C. B. translation.

4. E. Ch. Pukhal'skaya, Byull. Éksp. Biol. Med. 45, 3, 97 (1958).‡
5. K. D. Bock, H. Dengler, H. M. Kuhn, et al., Arch. Exper. Pathol. and Pharmacol. 230, 257 (1957).
6. V. Erspamer, Pharm. Rev. 6, 425 (1954).
7. V. Erspamer, Naunyn-Schmiedeberg's Arch. Exptl. Pathol. and Pharmacol 218, 1 2, 92 (1953).
8. H. Herxheimer, J. Physiol. (London) 122, 1, 49, (1953).
9. K. G. Scott, R. R. Scheline and R. S. Stone, Cancer Res. 18, 8, 927 (1958).
10. K. G. Scott and R. S. Stone, Cancer Res. 19, 7, 783 (1959).

‡Original Russian pagination. See C. B. translation.

THE MIGRATION OF Sr^{90} ALONG THE BIOLOGICAL CHAIN PLANT-TO-SHEEP-TO-FETUS

L. A. Buldakov and Yu. I. Moskalev

(Presented by Active Member AMN SSSR V. N. Chernigovskii)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 111-113, October, 1960
Original article submitted October 13, 1959

On account of the contamination of the earth's surface with long-lived products of uranium fission (Sr^{90}, Cs^{137}, and Ce^{144}) as a result of the testing of nuclear and thermonuclear weapons, the problem of the migration of radioactive isotopes along various biological chains has taken on great importance [3, 5–8].

Radioactive isotopes that fall to the earth's surface are incorporated into the general cycle of materials and quickly enter living organisms [1–3, 5, 6, 8, 11–15]. An object of particular concern is Sr^{90}, which has marked biological activity [10]. Studies of the accumulation of Sr^{90} in living organisms under conditions of prolonged intake from the surrounding environment have been relatively few in number, the majority having been made on small laboratory animals. So, for example, Comar, Whitney, and Lengemann [12] showed in experiments on rats that calcium is absorbed from the gastrointestinal tract to a greater extent than Sr^{90} (3.6 times greater), and accumulates to a greater extent in the fetal skeleton (5.1 times).

We have conducted an investigation on domestic animals (ewes). In this article we present the results of a study of the migration of Sr^{90} along the biological chain plant-to-sheep-to-fetus.

METHODS

The experiments were performed on 12 adult (2–4 years old) pregnant ewes, from which 23 fetuses were obtained weighing 200–300 g apiece. Over a six-month period the ewes were fed hay containing Sr^{90}. The specific activity of the hay was about 0.04 μC/kg weight. Six months after the experiment began, the animals were sacrificed. The content of Sr^{90} and stable calcium was determined in the skeletons of both the ewes and the fetuses (fetal ages 3–4 months). Samples for the measurement of radioactivity with an end-type counter (mass of mica window 2-3 mg/cm^2) were prepared from the ash that remained after incineration of the bones in a muffle furnace (at 500°). Weighed aliquots of the bone ash were transferred to special aluminum disks 38 mm in diameter. The activity of the samples was measured after radioactive equilibrium was established between Sr^{90} and its radioactive daughter isotope Y^{90}, and generally not less than 21 days after the animal was killed.

RESULTS

The data are presented in the table. Examination of the data shows that six months after the start of the experiments the concentration of radioactivity in the bones of the ewes (0.263 μC/kg weight) was approximately five times as high as in the hay (0.05 μC/kg weight), and 14.6 times as high as in the fetuses (0.018 μC/kg weight). This observation is in complete agreement with the results of studies made by E. B. Kurlyandskaya, N. L. Beloborodova, and E. F. Baranova [4], and by A. A. Rubanovskaya and V. F. Ushakova [9], with small laboratory animals. According to these authors' data, the concentration of Sr^{90} in the skeletons of rabbits and rats borne by mothers that had been fed Sr^{89} and Sr^{90} for a long time during pregnancy was only one-tenth as high as that of the mothers.

If the radioactivity is calculated per gram of Ca rather than per unit weight of bone, the Sr^{90} concentration of the fetal skeleton differs less strikingly from that of the mother.

In this case, as the table shows, the relative concentration of Sr^{90} in the maternal skeleton (0.00131 μC/g Ca) is only 2.3 times as great as that for the fetus (0.000565 μC/gCa). These data indicate that the skeleton of the mother "protects" the fetus from excessive entry of Sr^{90} into it.

It is worth noting that as Sr^{90} migrates down the biological chain plant-to-ewe-to-fetus, the concentration of radioactive material (if we express the data in μC/g calcium) goes down. Thus, for example, the Sr^{90} concentration (in μC/g of calcium) in hay is 4.73 times as great as in the bones of the mother, and 10.9 times as great as in the bones of the fetus. These observations indicate that in the transport of Sr^{90} along the biological chain, discrimination occurs between Sr^{90} and calcium. The discrimination coefficient shows how far the concentration of Sr^{90} per g calcium is reduced in the transfer from one step in the food chain to another. It is important to know the discrimination coefficients in order

Characteristics of the Migration of Sr^{90} from Plants to the Skeletons of Animals and to Fetal Skeletons Via the Placenta

Material compared	Pregnant ewe	Fetus weighing 200—300 g
Radioactivity of food due to Sr^{90} (in μC/kg of hay)	0.05	–
Concentration of Sr^{90} (in μC/kg wet weight of organ) in bones six months after start of experiment	0.263± 0.03	0.018± 0.005
Concentration of calcium (in g/kg): in hay	8	–
in bones	200	31.8
Concentration of Sr^{90} (in μC/g Ca) in hay consumed by ewes	0.0062	–
Concentration of Sr^{90} (in μC/g Ca) in skeleton	0.00131	0.000565
Ratio of Sr^{90} concentration (in μC/g Ca) in hay to that in skeleton	4.73	10.9
Discrimination coefficient for the chain hay-to-bone	0.21	0.091

Note: The discrimination coefficient is the ratio of the Sr^{90} concentration(in μC/g Ca) in the skeleton to that in hay.

to calculate the possible content of Sr^{90} in foodstuffs and in human bones. The discrimination coefficient for the chain plant-to-maternal bone is roughly 0.21, and for the chain plant-to-fetal bone, about 0.091. These figures correspond rather well to the results of studies made by other authors on small laboratory animals [3, 11, 12]. These studies show that the discrimination coefficients for the transfer from food to bone are 0.57, 0.35, and 0.20 for rats, mice, and rabbits, respectively. From the data we have been considering here we may conclude that the discrimination coefficient is not very species-dependent.

The presence of a discrimination coefficient or protection coefficient (the inverse of the discrimination coefficient) different from unity indicates that the metabolism of Ca and Sr, two alkali-earth elements that are similar in their chemical properties, is not completely identical, despite their tendency to concentrate in bone tissue and their similar types of distribution. In this connection it should be remembered that during prolonged intake the accumulation factor (the ratio of the content of the element in the whole skeleton to the daily intake per os) is different for the three alkali-earth elements Ca, Sr^{90}, and Ra^{226}, which are selectively absorbed in the the skeleton; its value is 1000, 45, and 20, respectively, for these elements [7]. The problem for subsequent investigations will be not only to determine the magnitudes of discrimination coefficients and protection coefficients for various steps in biological chains, but also to discover the mechanisms underlying the differences in distribution of elements that are similar in chemical properties.

SUMMARY

When pregnant sheep are fed radioactive hay with a specific activity of 0.05 μC/kg body weight for a period of six months, the concentration of Sr^{90} in the skeleton reaches 0.263 μC/kg. This concentration is 14.6 times as high as the concentration in the skeleton of the fetus. During Sr^{90} migration within the biological chain, discrimination occurs between Sr^{90} and calcium. Discrimination coefficients for the chains plant-to-mother and plant-to-fetus are 0.21 and 0.091, respectively; the protection coefficients, which are the reciprocals of the discrimination coefficients, are 4.37 and 10.9, respectively.

LITERATURE CITED

1. D. I. Il'in, Yu. I. Moskalev, and A. I. Petrova, Atomnaya Énerg. 5, 2, 171 (1958).*
2. V. M. Klechkovsky and I. V. Gulyakin, Int. J. Appl. Radiat. 2, 234 (1957).
3. A. M. Kuzin, in: The Threat to Humanity From Nuclear Explosions [in Russian] (Moscow, 1957).
4. É. B. Kurlyandskaya, N. D. Beleborodova, and E. F. Baranova, in: Materials on the Toxicology of Radioactive Substances [in Russian] (Moscow, 1957) No. 1, p. 16.
5. A. V. Lebedinskii, Med. Radiol. 5, 22 (1957).
6. A. N. Marei, M. M. Saurov, and G. D. Lebedeva, Med. Radiol., 1, 69 (1958).
7. Yu. I. Moskalev, in: Abstracts of Papers on Radioactive Strontium [in Russian] (Moscow, 1959) p. 83.
8. A. A. Peredel'skii, Zhur. Obshch. Biol. 18, 1, 17 (1957).
9. A. A. Rubanovskaya and V. F. Ushakova, in: Materials on the Toxicology of Radioactive Substances [in Russian] (Moscow, 1957) p. 23.
10. V. N. Strel'tsova and Yu. I. Moskalev, in: Abstracts of the Conference on Remote Sequelae of Lesions Produced by the Action of Ionizing Radiation [in Russian] (Moscow, 1956) p. 41.

11. C. L. Comar, R. H. Wasserman, and M. M. Nold, Soc. Exper. Biol. and Med. 92, 859 (1956).
12. C. L. Comar, I. B. Whitney, and F. W. Lengemann Proc. Soc. Exper. Biol. and Med. 88, 232 (1955).
13. W. R. Eckelmann, J. L. Kulp, and A. R. Schulert Science 127, 266 (1958).
14. J. L. Kulp, A., A. R. Schulert, and E. J. Hodges, Science 129, 1249 (1959).
15. W. F. Libby, Science 123, 657 (1956).

*Original Russian pagination. See C. B. translation.

REGENERATION OF THE PAROTID GLAND IN THE RAT

A. G. Babaeva

Laboratory of Growth and Development (Head-Prof. L. D. Liozner) of the Institute of Experimental Biology (Dir.-Prof. L. N. Maiskii), AMN SSSR, Moscow

(Presented by Active Member AMN SSSR V. V. Parin)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 113-118, October, 1960

Original article submitted October 12, 1959

There have been too few studies of the regenerative capacity of the salivary glands, and the data obtained by different authors are quite contradictory. It is thought that the submaxillary gland regenerates well [3, 5, 6, 7, 8], while the parotid gland has poor regenerative capacity [2, 4]. According to a widely held opinion, the recovery of either type of gland occurs through branching of the duct, with the formation of atypical, functionally imperfect glandular tissue. Some authors, taking a different view of the capacity of these glands to regenerate, have been guided solely by the size of the newly formed part of the gland. In the regenerated submaxillary gland this part occupies a greater area, according to available descriptions, than it does in the parotid gland. In the latter, the branching of the duct was so slight in experiments on dogs [4] and cats [2] that the authors questioned the capacity of the parotid to regenerate.

In a study of the parotid gland of the guinea pig [1] we found that the part of the gland formed as a result of atypical branching of the duct does in fact occupy a small area (at most 1/6) in relation to the area of the whole gland. We concluded, however, that the parotid possesses excellent regenerative capacity, since after removal of a large part of the gland its weight returns completely to normal, as a result of an increase in the size of the part that remains, which retains its normal structure for the most part. Thus, regeneration of the parotid gland of the guinea pig, like that of many other mammalian organs, comes about by regeneration hypertrophy – in this case, proliferation of the secretory cells of the acini over the entire organ, and not just near the site of injury. This proliferation ensures an increase in mass in a gland with normal structure, and this increase is the main mechanism by which the gland's weight is restored. Atypical branching of the duct is not of great importance in the regeneration of the organ as a whole. It might be supposed that the disagreement between our data on the mode and origin of salivary gland regeneration and the data of other authors depends on the fact that they adhered to classical concepts of the course of regeneration from the injured surface and paid most of their attention to that. Besides, other papers contain no quantitative account of the restoration of the mass of the organ, or of data on the dimensions of the region with atypical structure, which is evidently why the processes of regeneration hypertrophy escaped the authors' attention. But it is not impossible that the difference in results was caused by our use of a different species of animal from the one used by other authors, and by the fact that parotid rather than submaxillary regeneration was studied.

The present investigation was intended to show whether recovery by regeneration hypertrophy is peculiar to the salivary gland of guinea pigs or is a property of the salivary glands of other animals as well.

METHODS

As the object of this investigation, we used the parotid gland of the white rat, which is histologically a serous gland, like the parotid of the guinea pig, but is more compact and has a distinct connective tissue capsure. Two series of experiments were performed. In the first series we used 64 rats weighing from 60 to 100 g; in the second series we studied 14 adult rats weighing from 140 to 180 g. In all experiments, regeneration was studied after complete removal of the left parotid and extirpation of most of the right one. This arrangement was dictated by the need for obtaining results on rats that could be compared with the results of our experiments on guinea pigs. The importance of removing the contralateral gland is still not completely clear, since processes of compensatory hypertrophy do not occur in the salivary glands [7, 9, 10]. In the second series of experiments the animals were divided into two groups. In one group the capsule was sutured together after part of the gland was removed; in the other group only the

TABLE 1. Changes in Body Weight and Parotid Gland Weight in Rats with the Left Parotid Gland Removed (Supplementary Control)

No. of rat	Wt. of rat at start of expt. (g)	Weight of gland removed		Wt. of rat at end of expt. (g)	Weight of remaining gland		Weight gain (%)	
		absolute (mg)	relative (%)		absolute (mg)	relative (%)	rat	gland
1	62	32	0.516	174	80	0.459	180	150
2	67	40	0.597	166	73	0.439	147	82
3	78	39	0.500	163	70	0.429	108	72
4	86	38	0.441	218	99	0.454	153	160
5	93	41	0.440	209	74	0.354	124	80
6	100	41	0.410	194	69	0.355	194	168
Mean	81	38.5	0.484	187	77.5	0.415	134	102

muscle and skin were sutured. The animals in the first series were sacrificed two months after the operation; those in the second series, 1½ months after the operation. The glands were weighed, fixed in 10% formalin, and subjected to the usual histological treatment. Serial sections were stained with Gomori's stain and with hematoxylin and eosin.

RESULTS

First series. Young, rapidly growing rats were used in these experiments. In order to differentiate growth from regeneration, we sacrificed normal rats of the same age as those in the experiment at the beginning and the end of the experiment (there were seven rats in the initial control group, and 15 in the final one). Besides this, an additional control was set up to enable us to observe changes in body weight and gland weight in single animals. From the rats in this group we removed only the left parotid, leaving the other intact. This series was performed because of the published data, mentioned earlier, suggesting that processes of compensatory hypertrophy are absent from salivary glands.

According to the weight data from the initial and final controls, the difference in weight between the right and left parotid glands was insignificant; in the initial control it was not more than 4 mg, or 5%. Over a two-month period the body weight of the control rats increased by 97% on the average, and the weight of the parotid gland increased by 74%. This lag in the weight gain of the gland as compared to the body weight gain has the result that the relative weight of the organ has a tendency to decrease with age (from 0.474 to 0.427%). A similar pattern appeared in the supplementary control (Table 1): while the body weight increased 134% on the average, the weight of the gland increased by only 103%, resulting in a reduction of the relative weight from 0.484 to 0.415% during the two-month period. But the relative weight of glands in the rats of the supplementary control was no greater than that in the final control. This agrees with published data concerning the absence of pronounced compensatory hypertrophy in salivary glands. Data obtained on the control animals show that the animals' growth is accompanied by an increase in organ weight, during the two-month period, of roughly 74 – 103%. Since the weight of the glands in the experimental animals increased more during the same length of time, the difference must be attributed to restorative processes.

Analysis of the results obtained in the first series of experiments reveals that in individual rats the reaction to trauma was different, to judge from the change in the weight of the resected gland and the histological structure of these glands. On this basis the experimental rats can be divided into four groups.

In the rats of the first group (eight animals) the gland weight fell sharply during the experimental period The lumina of the acini of these glands were markedly enlarged as a result of flattening of the secretory epithelial cells. The nuclei of the epithelial cells were pycnotic. The gland had completely lost its specific form and had taken on the form of a system of excretory ducts, such as is characteristic for atrophying glands. The weight of the glands in rats of the second group (five animals) did not change during the experiment – that is, together with the weight of the removed portion it was approximately equal to the weight of the gland that had been removed intact. Degenerative changes were not so pronounced in these glands as in the previous group; the gland had not lost its specific form. In the animals of the third group (16 rats) the gland weight increased during the experiment, but this increase was either parallel with the gain in body weight or only a little greater. In the fourth group (Table 2) there were seven rats whose glands either weighed as much as those of the controls or else gained weight much faster than body weight increased in the experimental animals. It can be seen from Table 2 that with an average of 65%

TABLE 2. Changes in the Weight of theParotid Gland in Animals of the Fourth Group in the First Series

No. of rat	Wt. of rat at start of expt. (g)	Weight of glands removed (mg)		Wt. of rat at end of expt. (g)	Weight of regenerated gland	
		intact left gland	portion of right gland		absolute (mg)	relative (%)
1	71	43	25	181	56	0,309
2	92	40	28	270	95	0,351
3	92	36	25	187	67	0,358
4	98	34	29	171	55	0,321
5	99	40	30	205	43	0,209
6	111	53	26	269	95	0,359
7	112	53	32	201	55	0,273
Mean	96	42,7	27,8	212	66,5	0,311

TABLE 3. Changes in the Weight of the Parotid Gland in Animals of the First and Second Groups of the Second Series

No. of rat	Wt. of gland removed(mg)	Portion wt. of gland removed (mg)	Wt. of rat at end of expt. (g)	Wt. of regenerating gland	
				absolute (mg)	relative (%)
First group					
1	32	25	170	20	0,117
2	45	10	194	40	0,206
3	50	30	177	32	0,180
4	50	32	194	29	0,149
5	66	30	187	28	0,149
Mean	48,6	25,4	184	25,8	0,160
Second group					
1	34	21	204	57	0,279
2	43	20	169	57	0,337
3	—	—	195	45	0,230
4	46	24	192	44	0,228
5	62	26	195	40	0,205
Mean	46,2	22,7	191	48,6	0,255
Control					
				Right / Left	Right / Left
1	—	—	234	55 / 55	0,234 / 0,234
2	—	—	210	73 / 75	0,347 / 0,357
3	—	—	211	82 / 82	0,389 / 0,389
4	—	—	235	65 / 78	0,276 / 0,331
Mean	—	—	222	68,7 / 72,5	0,311 / 0,327

of the mass of the gland removed, the remainder grew 240% while the body weight increased only 120%, whereas in the control group, on the other hand, the gland gained weight somewhat more slowly than the body as a whole.

On the basis of these calculations, the presence of parotid gland regeneration cannot be questioned, although in several cases it cannot be considered complete, since complete restoration of its weight was not observed and the mean values of the absolute and relative weight are 80.1% and 72.8%, respectively, of the mean weight of glands of control animals at the end of the experiment. At histological examination it was found that in the glands of rats in the third group, which were mainly normal in structure, there were regions of marked vacuolization of the protoplasm of secretory epithelial cells.

In the fourth group of rats, the glandular tissue has little to distinguish it, with the stains used, from the glandular tissue of control rats; it retains the variations in the dimensions of nuclei in the secretory epithelium, in the size of the acini, and in the number of secretory cells constituting an acinus, that are characteristic of parotid glands in the rat. The regenerating gland differs from the normal in that it has a small connective tissue scar, and in some cases (as in the third group) a very narrow strip of glandular tissue of atypical structure at the site of amputation. Even in those cases where such regions of atypical structure are present, they occupy an insignificant fraction of the area of the section (a few percent), so that they do not appear to play any major role in the increase in gland weight. This increase results from proliferation of the cells of the secretory epithelium (regeneration hypertrophy), as is indicated by the normal histological structure of the great bulk of the gland, the absence of atypical branching of the duct in several regenerated glands whose weight was close to that of the glands in control animals, and the large number of mitoses in the secretory cells (2.3 mitoses per thousand cells), which remained for as long as two months after the operation in some instances. In the control glands the number of mitoses did not exceed 0.6 per thousand cells.

Second series. The results of the first series of experiments showed that regeneration of the parotid gland of young rats can occur, but restoration occurred in only 19% of the cases, i. e., in a much smaller number of cases than with guinea pigs. We conjectured that the presence of a distinct connective tissue capsule in rats might have an effect on salivary gland regeneration. To test this hypothesis, we did not suture the capsule after the operation in some of the animals of the second series. In addition, in the second series we set out to determine whether regeneration of the parotid can occur in sexually mature rats growing more slowly than young rats. The results of these experiments are shown in Table 3. The difference between the two groups of animals was distinct. In the cases where the capsule was not sutured (second group), the increase in the weight of the resected gland $1\frac{1}{2}$ months after operation was greater. The increase in gland weight among animals in the first group was insignificant. The differences between the two groups of the second series were statistically significant ($P = 0.01$). As in the first series, in individual rats of the second group complete recovery of the entire traumatized organ occurred after removal of 50% of the gland; but the mean value of the relative weight was also somewhat below that for the control animals, being 82% of the latter. Upon histological examination of the regenerated glands, data were obtained that were similar to those described for the fourth group of rats in the first series. Thus, in rats, as in guinea pigs, regeneration of the parotid gland occurs by regeneration hypertrophy, but in contrast to guinea pigs, growth from the injured surface with the formation of atypical glandular tissue is much less marked.

SUMMARY

Parotid gland regeneration was studied in rats in which all of the left gland and part (50 – 60%) of the right had been removed. In young rats weighing 60 – 100 g, regeneration occurred in 19% of cases, as judged by the restoration of the weight of the organ. The weight of the glands did not always reach the initial level, however, and averaged 80% of the weight in control animals.

The number of cases with gland regeneration increased in adult rats when the capsule was left open after the operation. Histological investigation showed that regeneration does not occur by growth starting at the injured surface, but rather by the proliferation of the secretory epithelium (regenerative hypertrophy) of the acini over the whole organ.

LITERATURE CITED

1. A. G. Babaeva, Byull. Éksp. Biol. Med. 43, 3, 95 (1957).*
2. E. Sh. Gerlovin, Abstracts of Ukrainian Conference of Morphologists [in Russian] (Kharkov, 1956) Vol. 2, p. 51.
3. E. Sh. Gerlovin, Transactions of the Leningrad Sanitary Hygiene Medical Institute [in Russian] (1958) Vol. 42, p. 132.
4. V. M. Koropov, Materials on the Pathological Physiology of the Salivary Glands [in Russian] (Moscow, 1949).
5. W. Podwyssozki, Beitr. path. Anat. 2,19 (1887).
6. A. Carraro, Frankf. Z. Path. 3, 26 (1909).
7. B. B. Milstein, J. Exper. Path. 31, 664 (1950).
8. Ribbert, Arch. Entwicklungs Mechan. 6, 537 (1898).
9. E. H. Schäfer and B. Moor, J. Physiol. 19, 13.†
10. H. Teir, Acta path. et microbiol. scandinav. 32, 337 (1953).

* Original Russian pagination. See C. B. translation.

† No year given in original - Publisher.

24-HOUR RHYTHM OF CELL MULTIPLICATION IN RAT EPIDERMIS DURING HEALING OF SKIN WOUNDS

M. T. Gololobova

Laboratory of Histophysiology (Head-Candidate of Biological Sciences V. N. Dobrokhotov) of the Institute of Experimental Biology (Dir.-Prof. I. N. Maiskii) of the AMN SSSR, Moscow

(Presented by Active Member AMN SSSR N. N. Zhukov-Verezhnikov)

Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50, No. 10, pp. 118-122, October, 1960

Original article submitted January 22, 1960

It is known from numerous observations that in the tissues around a wound defect the number of cell divisions increases greatly, to an extent which depends on the size of the wound inflicted and the species of experimental animal. But the question of the 24-hour rhythm of mitoses in tissues during wound healing has been very inadequately studied, and the available data are contradictory.

Blumenfeld [8] investigated the 24-hour rhythm of cell division in the epidermis during healing of skin wounds in rabbits. Each animal received six wounds at intervals of four hours. Mitotic activity was studied on the seventh day of wound healing. On the basis of the data so obtained Blumenfeld concluded that in the epidermis of a healing wound in the rabbit, there is a 24-hour rhythm in mitotic activity, the mean number of mitoses being twice as great in the regenerating epidermis as in the normal skin. Bullough and Laurence [9] showed that if six small radial wounds are made simultaneously on one ear of a mouse, mitotic activity in the epidermis next to the wounds is greatest on the third or fourth day of regeneration. They were not able to detect a 24-hour rhythm in the mitotic activity. The design of these experiments, which involved making several wounds in each animal, merits critical examination, since other data indicate that a regeneration process in one part of an organism has an effect on mitotic activity in other tissues—even those at a distance [3].

The disagreement in the published data concerning a 24-hour rhythm of mitotic cell division in regenerating tissues led us to carry out the present investigation. We set ourselves the problem of finding out, first, how the level of mitotic activity in the epidermis changes from one period to the next during the healing of skin wounds, and second, whether there is a 24-hour rhythm of cell multiplication in the epidermis adjacent to the wound defect, and in the regenerating epidermis – i.e., in the cell layer that is forming a cover over the wound surface.

METHODS

Experiments were performed on 100 rats (male) weighing 150 g. A wound (3 × 8 mm) was made on the skin of the back at 9:00 AM. The skin was removed together with the subcutaneous connective tissue. The first eight skin biopsies removed were fixed for the purpose of studying mitotic activity in the normal epidermis; a second group of biopsies (also eight in number) were fixed at 9:00 PM for the same purpose. For studies of mitotic activity during regeneration, skin biopsies were taken every six hours on the first, third, and fifth day, in such a manner that portions of both regenerating epidermis and epidermis adjacent to the wound were included (the dimensions of biopsies from each side of the wound were 5 – 7 mm). Mitotic activity was determined separately for each of these biopsies. Seven animals were used for each time period. The material was fixed in Zenker's solution and imbedded in paraffin. Sections 7 μ in thickness were stained with hematoxylin by Karazi's method.

RESULTS

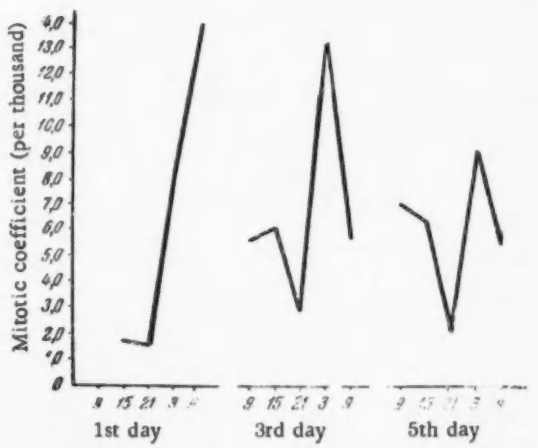
The figure shows the changes in the mitotic coefficient (MC) in epidermis adjacent to the wound at various times of the day. At 9 AM (before the wound was made) the mitotic coefficient had a value of 4.9 per thousand, and at 9 PM this coefficient was 2.1 per thousand.

Data obtained during the first day of the regeneration process show that at 3 PM, i.e., six hours after the wound was made, the number of cell divisions in the epidermis next to the wound had fallen considerably, to a level (MC = 1.6 per thousand) comparable to that observed in normal rat epidermis at the hours of lowest mitotic activity. At 9 PM, i.e., 12 hours after the wound

was made, the level of mitotic activity was almost the same as it was in control animals at the same time of day. At 3 AM the number of mitoses had risen greatly, and it continued to rise until it reached a maximum at 9 AM. The changes in the mitotic coefficient between 9 PM and 3 AM and between 3 AM and 9 AM were statistically significant (P= 0.0001 and P = 0.02).

We have shown previously [1] that in the normal rat epidermis the 24-hour rhythm of cell division can be expressed by a curve with a single peak, with the maximum number of mitoses in the early hours (6 AM) and the minimum at 9 PM. In our experiment the mitotic activity continued to increase during the first day of the regeneration process until 9 AM. This increase in the number of mitoses toward the end of the first 24 hours of the regeneration process can be explained by a compensatory increase in mitotic activity in response to inhibition of this activity.

At the beginning of the third day of regeneration (9 AM) mitotic activity in the epidermis adjacent to the wound was not significantly different from what was observed at 9 AM in the normal skin. The change in the mean MC during the interval between 9 AM and 3 PM also was not significant. After 3 PM we noted a reduction in the number of mitoses, but this reduction was not significant (P = 0.16). Then, after 9 PM the level of mitotic activity in the old epidermis increased so sharply that at 3 AM of the third day of regeneration we were able to establish the largest number of mitoses, differing significantly from the number at 9 PM (P = 0.0001). At 9 AM at the end of the third day of regeneration we observed a fall in the number of mitoses to a value, like that at 9 AM at the beginning of the same day, only a little greater than in the normal epidermis. The change in the MC between 3 AM and 9 AM was statistically significant (P = 0.001). Therefore, on the third day of



24-hour changes in the mitotic coefficients (MC) in epidermis during healing of skin wounds.

Change in the MC in Regenerating Epidermis on the Third and Fifth Days of Healing of Skin Wounds

Regeneration time	Time of day					Mean for the day
	9 AM	3 PM	9 PM	3 AM	9 AM	
3rd day	5,7	4,3	7,2	7,2	5,9	6,1
5th day	4,3	5,5	3,2	4,6	4,6	4,4

healing the epidermis adjacent to the wound is characterized by the presence of a 24-hour rhythm of cell multiplication that takes the form of a curve with a single maximum of the number of mitoses at 3 AM and a minimum at 9 AM.

At 9 AM, at the beginning of the fifth day, the level of mitotic activity in the epidermis was a little higher than at the same time in normal skin; but statistical analysis indicated that this difference was not significant (P = 0.04). Starting at 3 PM, the number of mitoses in the epidermis decreased, reaching a minimum at 9 PM. The difference in the number of mitoses between 3 PM and 9 PM was statistically significant (P = 0.002). At 3 AM the level of mitotic activity reached the highest point, and was significantly different from the level at 9 PM (P = 0.001). The reduction in the number of mitoses by 9 AM at the end of the fifth 24-hour period was considerable, but not significant (P = 0.04). It follows that on the fifth day of the regeneration process the epidermis next to the wound is also characterized by the presence of a 24-hour rhythm in cell multiplication, but the total level of mitotic activity is somewhat lower than on the third day of healing of skin wounds.

What sort of mitotic activity takes place in the regenerating epidermis? Since epithelization of the wound surface did not occur on the first day after the wounds were made, we began examining mitotic activity on the third day. On the third day of regeneration, we found tongues of regenerating epidermis under the scab, growing in from the margins of the old epidermis. But the regenerating epidermis covers only about one third of the whole surface of the wound at this time. On the fifth day the degree of epithelization of the wound surface is different: in some cases epithelium covers half the surface of the wound, while in others complete epithelization of the wound surface is seen. No consistent changes are seen in the number of mitoses in the regenerating epidermis at different hours of the day on the 3rd-5th day of regeneration. In other words, we have not been able to detect a 24-hour rhythm of cell multiplication in regenerating epidermis. We can only point out that the number of cell divisions on the third day of healing of skin wounds (mean MC = 6.1) is a little higher than on the fifth day (mean MC = 4.4; see table).

In view of work that has been done on reactive inhibition of mitoses [6] the impression may have been created that the level of mitotic activity in the animal organism is quite labile. It is true that in most cases such inhibition is of short duration, and the number of cell divisions quickly returns to normal. But the situation is somewhat different with the 24-hour rhythm of cell multiplication, in connection with which one might speak of a certain stability. To eliminate the 24-hour rhythm of mitoses or obtain an inversion of the rhythm, certain experimental conditions must be met [10].

We would like to call attention to the maintenance of the 24-hour rhythm of mitotic activity in the case of traumatic regeneration. Thus, for example, we have shown [3] that if a burn is produced on one cornea of the rat, the 24-hour rhythm of cell division is maintained in the other cornea. The 24-hour rhythm of mitotic activity also is seen distinctly in the liver of rats and mice after partial hepatectomy [5, 11].

Our results agree with those of Blumenfeld [8] concerning the presence of a 24-hour rhythm of mitotic activity in the epidermis during healing of skin wounds. But he examined the progress of regeneration in rabbit epidermis on the seventh day only, when the process of epithelization is over.

Our data on the absence of a 24-hour rhythm of mitotic activity in regenerating epidermis are of interest. V. N. Dobrokhotov [3] has also noted that in regenerating epidermis the level of mitotic activity at all periods during the healing of skin wound is almost the same as in the epidermis of normal skin, which agrees with the results of our experiments on the 3rd-5th days of regeneration.

Results similar to ours, though not directly concerned with the 24-hour rhythm, were obtained by G. A. Kovaleva [4], who showed that the response of regenerating tissue to stimulation of the animals with an electrical current is different from that in the adjacent epithelium: whereas a marked depression is seen in the rate of cell division in the epithelium adjacent to the wound (cornea, tongue), in the regenerating epithelial layer reactive inhibition is almost absent (tongue). At the end of the regeneration process the reactivity of the epithelium becomes normal.

The difference that we found between the levels of mitotic activity on the third and fifth days of regeneration appeared only at the time of the maximum number of cell divisions (3 AM), for the most part. A similar phenomenon has been reported by other authors [2, 7, 12]. For this reason, it is recommended that materials be fixed in the early morning hours to obtain more clear-cut differences between experimental and control groups.

SUMMARY

Observations revealed that cell multiplication follows a 24-hour rhythm in the epidermis adjacent to a skin wound on the rat's back. The greatest differences between the mitotic activities at different stages of wound healing (on the first, third, and fifth day of regeneration) were observed at the time when the number of cell divisions was maximal, i. e., in the early morning hours. No 24-hour rhythm was seen in the mitotic activity of regenerating epidermis directly over the wound.

LITERATURE CITED

1. M. T. Gololobova, Byull. Éksp. Biol. Med. 45, 9, 118 (1958).*
2. M. T. Gololobova, Byull. Éksp. Biol. Med. 47, 3, 94 (1959).*
3. V. N Dobrokhotov, Abstracts of the Sixth All-Union Congress of Anatomists, Histologists, and Embryologists [in Russian] (1958) p. 515.
4. G. A. Kovaleva, Byull. Éksp. Biol. Med. 47, 2, 116 (1959).*
5. L. D. Liozner, Z. A. Ryabinina, and V. F. Sidorova, Byull. Éksp. Biol. Med. 47, 5, 96 (1959).*
6. G. S. Strelin and V. V. Kozlov, Arkh. Anat. Gistol. i Émbriol. 36, 2, 3 (1959).
7. I. A. Utkin, Voprosy Onkol. 1, 4, 3 (1955).
8. C. M. Blumenfeld, Archive of Pathology 36, 5, 493 (1943).
9. W. S. Bullough and E. B. Laurence, Br. Journal of Experimental Pathology 38, 3, 273 (1957).
10. F. Halberg, C. P. Barnum, R. H. Silber, and J. J. Bittner, Proc. Society for Exper. Biol and Med. 97, 897 (1958).
11. J. J. Jaffe, Anatomical Record 120, 4, 935 (1954).
12. Ra. Vasama and Ri. Vasama, Annales Med. exper. et biol. fenniae 35, 363 (1957).

*Original Russian pagination. See C. B. translation.

DEVELOPMENT AND REGRESSION OF HEXESTROL-INDUCED CHANGES IN THE SPERMATIC TRACT OF THE RABBIT

A. G. Bobkov

Department of Pathological Anatomy (Head-Prof. M. A. Zakhar'evskaya)
of the I. P. Pavlov First Leningrad Medical Institute
(Presented by Academician N. N. Anichkov)
Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 50,
No. 10, pp. 122-126, October, 1960
Original article submitted October 26, 1959

In experimental studies of changes in the male sexual apparatus under the influence of estrogens, only a few authors have given particular attention to the spermatic tracts. Atrophy of the epithelium in the epididymis and in the vas deferens has been described. Edema of the mucosa [1] and hypertrophy of the fibromuscular tissue [4] have also been reported; the latter, however, is not always observed, and depends on the period of sexual activity. T. Martins and J. R. Valle [2] found that estrogen in vitro enhances the motor activity of the vas deferens. H. Burrows [1] found scattered instances of planocellular metaplasia with keratinization in the epididymis in mice. Great interest attaches to the report of Vazquez-Lopez concerning two instances of a small carcinoma of the epidymis and one case of hyperplasia of the head of the epididymis occurring in hamsters as a result of prolonged treatment with estrogens. Unfortunately, the author [3] did not give data on the histological examination.

METHODS

Observations were conducted on rabbits weighing 2–2.5 kg. All the animals were injected subcutaneously with hexestrol in a 2% solution in oil at the rate of 0.45 ml per animal (0.009 g of hexestrol) three times a week. In the first series (24 animals) the duration of the experiments was from 3 to 311 days, hexestrol being injected regularly until the end of the experiments. In the second series (17 animals) hexestrol was injected for the first three months, after which the injections were stopped so that the regression of hexestrol-induced changes could be studied. The experiments lasted for from 30 to 464 days after the injections were stopped.

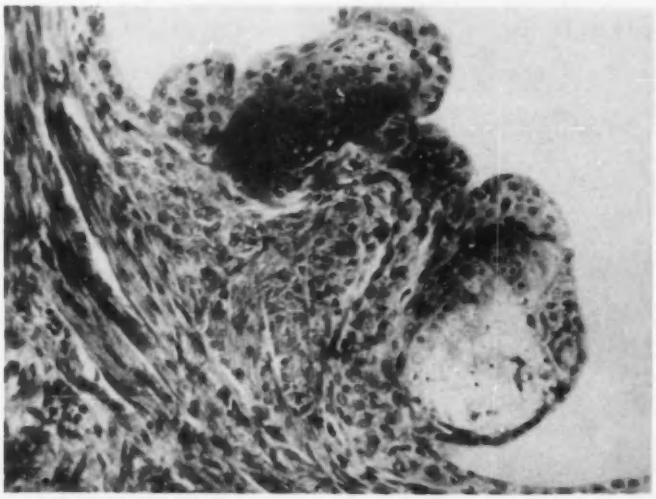


Fig. 1. Focal proliferation of epithelium with atypical keratinization in tubules of the tail of the epididymis. Experiment on 71st day of hexestrol administration (total amount injected, 0.279 g). Stained with hematoxylin and eosin. Ocular 4, objective 8. Magnification 250 ×.

For the histological examination, tissue biopsies were fixed in 10% formalin, imbedded in celloidin-paraffin, and stained with hematoxylin and eosin and with van Gieson's stain. In some instances, staining with toluidine blue by the method of Gram, Mallory, and Heidenhain was also employed. Various portions of the spermatic tracts and testes were subjected to examination.

RESULTS

Under the influence of hexestrol, atrophy develops in the testes during the first 2-3 months; spermatogenesis stops, and the cells of the spermatogenic epithelium degenerate, along with some of the Sertoli cells; the convoluted seminiferous tubules diminish markedly in size. In the stroma, the number of interstitial cells increases. Testicular atrophy continues as long as hexestrol is injected.

Increasing signs of atrophy are also observed in various divisions of the spermatic tract during this period. It is only in the tubules of the rete testis, whose structure is very simple, that no perceptible changes occur. The cilia disappear from the ductuli efferentes testis, and the stereocilia from the tubules of the ductus epididymidis. The epithelium becomes smooth, and the cells decrease in size. The excretion of secretory granules stops. In the lumen, there is a decrease in the number of spermatozoa and desquamated cells of spermatogenic epithelium; the diameter of the tubules is reduced. In the vas deferens and the ejaculatory duct there is also a reduction in the size of the epithelial cells and a contraction of the lumen. In some cases one sees a considerable amount of karyorrhexis of epithelial cells in the ductus epididymidis and the ampulla of the vas deferens; in the ampulla and the ejaculatory duct edema is also found, and a moderate infiltration of the stroma and epithelium with lymphocytes and pseudoeosinophils. In the tubules of the tail of the epididymis, mild proliferation is observed here and there, and thickening of the epithelial layer without definite differentiation.

In two cases (after 71 and 180 days) we found multiple small foci of an unusual type of stratified squamous epithelium with atypical keratinization in the tubules of the ductus epididymidis, in the region where it passes over into the first part of the vas deferens (Fig. 1). In the early stages of development these foci are limited proliferations in the form of intraepithelial nests of tiny cells with a small amount of basophilic protoplasm and a dark round nucleus. These cell clusters protrude into the lumen. A layer of dedifferentiated cells of pre-existing epithelium frequently remains over them. Later, a palisade-like layer of cells with a slightly elongated form appears at the base of the cluster. Mitoses are found in these cells. In the central and upper layers of the epithelial cell proliferation, the cells become larger and the protoplasm lighter, while the nuclei become pycnotic or undergo karyorrhexis. As a result the cells change into non-nucleated lamellae forming stratified layers. Some of these are stained shades of blue-violet with Gram's stain. In the tubule membrane proper, mild lymphocytic infiltration is often noted near the epithelial proliferations. thelium with mitoses in individual cells. In the same experiment, focal proliferation of stratified squamous epithelium with atypical keratinization was found in the ampulla of the vas deferens, but in two other experi-

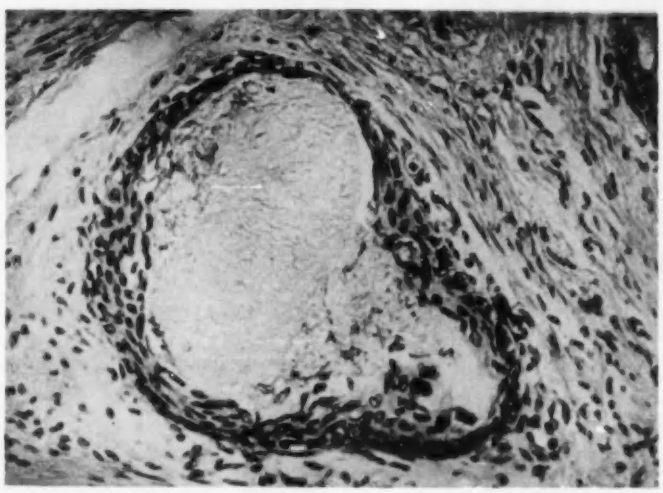


Fig. 2. Keratinization and desquamation of epithelium in tubule of the tail of the epididymis. Experiment on 50th day after termination of hexestrol administration. Stained by van Gieson's method. Ocular 4, objective 8. Magnification 250 x.

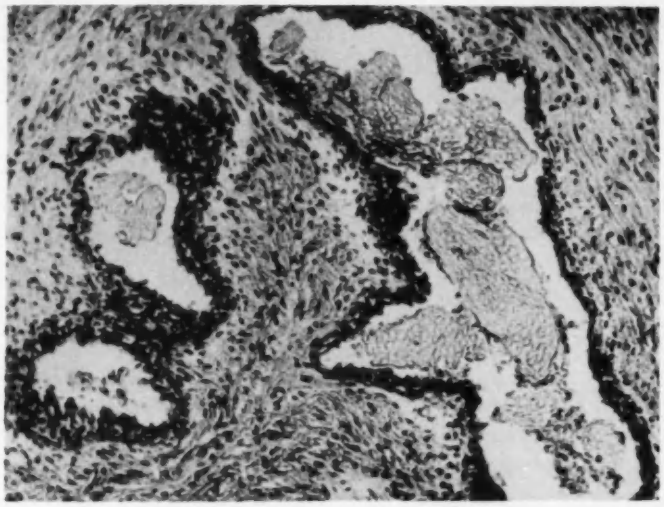


Fig. 3. Desquamated keratinized sheets in tubules of the tail of the epididymis. Normal differentiation of epithelium. Experiment on 270th day after termination of hexestrol administration. Stained by van Gieson's method. Ocular 4, objective 8. Magnification 250 ×.

After the injections of hexestrol are stopped, the atrophy and collapse of the convoluted tubules in the testes remain for several months. But by the end of the second month infrequent mitoses appear in the epithelial cells. In some animals mitoses appear in large numbers after four months, while in others proliferation occurs later. After eight months spermiogenesis is restored, the convoluted tubules grow to their normal size, and the number of interstitial cells decreases. In the spermatic tracts, pronounced atrophy and collapse of the tubules remains for a long time. But after 5–6 months the epithelium gradually becomes thicker, and mitoses are encountered infrequently in the epithelial cells; after seven months cilia appear in the ductuli efferentes; after nine months stereocilia are seen in the ductus epididymidis, and the lumen of the ducts is filled with spermatozoa and desquamated epithelial cells. The ampullae of the vasa deferentia are markedly dilated, and the epithelium becomes flat as it undergoes histological accommodation. In the ductuli efferentes one often encounters multiple small intraepithelial cavities as late as $5\frac{1}{2}$ months; these are the result of cell degeneration. In one experiment 50 days after hexestrol injections were stopped, stratified squamous epithelium with keratinization and desquamation of cells was found (Fig. 2). In other parts of the duct we encountered regions of thickened epithelium with indications of vertical anisomorphism, but with pycnotic nuclei and desquamation of individual cells, and at the base of the layer there was differentiation of the epiments (at 30 and 270 days; Fig. 3) desquamated cornified sheets and scales were found in the lumen of tubules in the tail of the epididymis; the wall of these tubules was covered with epithelium consisting of one or two rows of cells. These changes in the condition of the structures of the multilayered flattened epithelium enable us to say that the epithelial metaplasia is of temporary and transient character.

SUMMARY

The author studied the changes that occur in the spermatic tract of rabbits during and after the administration of hexestrol. Under the influence of hexestrol administration lasting for from 3 to 311 days, atrophy of the epithelium occurs along with partial collapse of various portions of the duct system, as well as edema and moderate cellular infiltration of the distal portion of the duct system. Metaplasia of the epithelium into the stratified squamous type, with atypical keratinization, was observed in the distal portion of the ductus epididymidis in 5 out of 41 animals; in one rabbit the ampulla of the vas deferens was also involved. From two to seven months after the cessation of hexestrol administration (which was continued for three months in this group) a slow restoration of specific differentiation occurred. The dependence of atrophic and regenerative changes in the vas deferens on similar changes in the testes is emphasized.

LITERATURE CITED

1. H. Burrows, J. Path. and Bact. 41, 423 (1935).
2. T. Martins and J. R. Valle, Compt. rend. Soc. Biol. 129, 1122 (1938).
3. E. Vazquez-Lopez, J. Path. and Bact. 56, 1 (1944).
4. L. J. Wells, Anat. Rec. 64, 475 (1936).

ABBREVIATIONS MOST FREQUENTLY ENCOUNTERED IN RUSSIAN BIO-SCIENCES LITERATURE

Abbreviation (Transliterated)	Significance
AMN SSSR	Academy of Medical Sciences, USSR
AN SSSR	Academy of Sciences, USSR
BIN	Biological Institute, Botanical Institute
FTI	Institute of Physiotherapy
GONTI	State United Sci-Tech Press
GOST	All Union State Standard
GRRRI	State Roentgenology, Radiology, and Cancer Institute
GTTI	State Technical and Theoretical Literature Press
GU	State University
I Kh N	Scientific Research Institute of Surgical Neuropathology
IL (IIL)	Foreign Literature Press
IONKh	Inst. Gen. and Inorganic Chemistry (N. S. Kurnakov)
IP	Soil Science Inst. (Acad. Sci. USSR)
ISN (Izd. Sov. Nauk)	Soviet Science Press
Izd.	Press
LEM	Laboratory for experimental morphogenesis
LENDVI	Leningrad Inst. of Dermatology and Venereology
LEO	Laboratory of Experimental Zoology
LIKhT	Leningrad Surgical Institute for Tuberculosis and Bone and Joint Diseases
LIPZ	Leningrad Inst. for Study of Occupational Diseases
LIPK	Leningrad Blood Transfusion Institute
Medgiz	State Medical Literature Press
MOPISh	Moscow Society of Apiculture and Sericulture
MVI	Moscow Veterinary Institute
MZdrav	Ministry of Health
MZI	Moscow Zootechnical Institute
LOKhO	Leningrad Society of Orthopedic Surgeons
NIIZ	Scientific Research Institute of Zoology
NINKhI	Scientific Research Institute of Neurosurgery
NIU	Scientific Institute for Fertilizers
NIUIF	Scientific Research Institute of Fertilizers and Insecticides
NIVI	Veterinary Scientific Research Institute
ONTI	United Sci.-Tech. Press
OTI	Division of Technical Information
RBO	Russian Botanical Society
ROP	Russian Society of Pathologists
SANIIRI	Central Asia Scientific Research Institute of Irrigation
SANIISh	Central Asia Scientific Research Institute of Sericulture
TsNII	All-Union Central Scientific Research Institute
TsNTL	Central Scientific and Technical Laboratory
VASKhNIL	All-Union Academy of Agricultural Sciences
VIG	All-Union Institute of Helminthology
VIEM	All-Union Institute of Experimental Medicine
VIR	All-Union Institute of Plant Cultivation
VIUAA	All-Union Institute of Fertilizers, Soil Science, and Agricultural Engineering
VIZR	All-Union Institute of Medical and Pharmaceutical Herbs
VNIRO	All-Union Scientific Institute of Fishing and Oceanography
ZIN	Zoological Inst. (Acad. Sci. USSR)

Note: Abbreviations not on this list and not explained in the translation have been transliterated, no further information about their significance being available to us. - Publisher.

RUSSIAN JOURNALS FREQUENTLY CITED
[Biological Sciences]

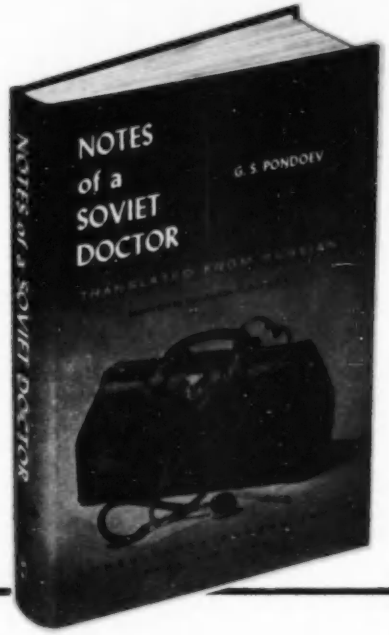
Abbreviation*	Journal*	Translation
Agrobiol.	Agrobiologiya	Agrobiology
Akusherstvo i Ginekol.	Akusherstvo i Ginekologiya	Obstetrics and Gynecology
Antibiotiki	Antibiotiki	Antibiotics
Aptechnoe Delo	Aptechnoe Delo	Pharmaceutical Transactions
Arkh. Anat. Gistol. i Émbriol.	Arkhiv Anatomii Gistologii i Émbriologii	Archives of Antomy, Histology, and Embryology
Arkh. Biol. Nauk SSSR	Arkhiv Biologicheskikh Nauk SSSR	Archives of Biological Science USSR
Arkh. Patol.	Arkhiv Patologii	Archives of Pathology
Biofizika	Biofizika	Biophysics
Biokhimiya	Biokhimiya	Biochemistry
Biokhim. Plodov i Ovoshchei	Biokhimiya Plodov i Ovoshchei	Biochemistry of Fruits and Vegetables
Bot. Zhur.	Botanicheskii Zhurnal	Journal of Botany
Byull. Éksptl. Biol. i Med.	Byulleten Éksperimentalnoi Biologii i Meditsiny	Bulletin of Experimental Biology and Medicine
Byull. Moskov. Obshchestva Ispytatelei Prirody, Otdel Biol.	Byulleten Moskovskogo Obshchestva Ispytatelei Prirody, Otdel Biologicheskii	Bulletin of the Moscow Naturalists Society, Division of Biology
Doklady Akad. Nauk SSSR	Doklady Akademii Nauk SSSR	Proceedings of the Academy of Sciences USSR
Éksptl. Khirurg.	Éksperimentalnaya Khirurgiya	Experimental Surgery
Farmakol. i Toksikol.	Farmakologiya i Toksikologiya	Pharmacology and Toxicology
Farmatsiya	Farmatsiya	Pharmacy
Fiziol. Rastenii	Fiziologiya Rastenii	Plant Physiology
Fiziol. Zhur. SSSR	Fiziologicheskii Zhurnal SSSR im. I. M. Sechenova	I. M. Sechenov Physiology Journal USSR
Gigiena i Sanit.	Gigiena i Sanitariya	Hygiene and Sanitation
Izvest. Akad. Nauk SSSR, Ser. Biol.	Izvestiya Akademii Nauk SSSR, Seriya Biologicheskaya	Bulletin of the Academy of Sciences USSR, Biology Series
Izvest. Tikhookeanskogo N. I. Inst. Rybnogo Khoz. i Okeanog.	Investiya Tikhookeanskogo N. I. Instituta Rybnogo Khozyaistva i Okeanografii	Bulletin of the Pacific Ocean Scientific Institute of Fisheries and Oceanography
Khirurgiya	Khirurgiya	Surgery
Klin. Med.	Klinicheskaya Meditsina	Clinical Medicine
Lab. Delo	Laboratornoe Delo (po Voprosam Meditsiny)	Laboratory Work (on Medical Problems)
Med. Parazitol.	Meditsinskaya Parazitologiya i Parazitarnye Bolezni	Medical Parasitology and Parasitic Diseases
Med. Radiol.	Meditsinskaya Radiologiya	Medical Radiology
Med. Zhur. Ukrain.	Medichnii Zhurnal Ukrainskii	Ukrainian Medical Journal
Mikrobiologiya	Mikrobiologiya	Microbiology
Mikrobiol. Zhur.	Mikrobiologichnii Zhurnal	Microbiology Journal
Nevropatol., Psikhyat. i Psikhogig.	Nevropatologiya, Psikhyatriya i Psikhogigiena	Neuropathology, Psychiatry and Psychohygiene
Ortoped., Travmatol. i Protez.	Ortopediya, Travmatologiya i Protezirovanie	Orthopedics, Traumatology and Prosthetics
Parazitol. Sbornik	Parazitologicheskii Sbornik	Parasitology Collection
Pediatriya	Pediatriya	Pediatrics
Pochvovedenie	Pochvovedenie	Soil Science
Priroda	Priroda	Nature
Problemy Éndokrinol. i Gormonoterap.	Problemy Endokrinologii i Gormonoterapii	Problems of Endocrinology and Hormone Therapy
Problemy Gematol.	Problemy Gematologii i Perelivaniya Krovi	Problems of Hematology and Blood Transfusion
Problemy Tuberk.	Problemy Tuberkuleza	Problems of Tuberculosis
Sovet. Med.	Sovetskaya Meditsina	Soviet Medicine
Sovet. Vrachebny Zhur.	Sovetskii Vrachebnyi Zhurnal	Soviet Physicians Journal
Stomatologiya	Stomatologiya	Stomatology

* BRITISH-AMERICAN TRANSLITERATION SYSTEM.

(continued)

(continued)

Abbreviation	Journal	Translation
Terap. Arkh.	Terapevticheskii Arkhiv	Therapeutic Archives
Trudy Gelmint. Lab.	Trudy Gelmintologicheskoi Laboratoriya	Transactions of the Helminthology Laboratory
Trudy Inst. Genet.	Trudy Instituta Genetiki	Transactions of the Institute of Genetics
Trudy Inst. Gidrobiol.	Trudy Instituta Gidrobiologiya	Transactions of the Institute of Hydrobiology
Trudy Inst. Mikrobiol.	Trudy Instituta Mikrobiologiya	Transactions of the Institute of Microbiology
Trudy Inst. Okean.	Trudy Instituta Okeanologiya, Akademii Nauk SSSR	Transactions of the Institute of Oceanology, Academy of Sciences, USSR
Trudy Leningrad Obshchestva Estestvoisp.	Trudy Leningrad Obshchestva Estestvoispytatelei	Transactions of the Leningrad Society of Naturalists
Trudy Vsesoyuz. Gidrobiol. Obshchestva	Trudy Vsesoyuznogo Gidrobiologicheskogo Obshchestva	Transactions of the All-Union Hydrobiological Society
Trudy Vsesoyuz. Inst. Eksptl. Med.	Trudy Vsesoyuznogo Instituta Eksperimentalnoi Meditsiny	Transactions of the All-Union Institute of Experimental Medicine
Ukrain. Biokhim. Zhur.	Ukrainskii Biokhimichnii Zhurnal	Ukrainian Biochemical Journal
Urologiya	Urologiya	Urology
Uspekhi Biokhimiya	Uspekhi Biokhimiya	Progress in Biochemistry
Uspekhi Sovremennoi Biol.	Uspekhi Sovremennoi Biologiya	Progress in Contemporary Biology
Vestnik Akad. Med. Nauk SSSR	Vestnik Akademii Meditsinskikh Nauk SSSR	Bulletin of the Academy of Medical Science USSR
Vestnik Khirurg. im. Grekova	Vestnik Khirurgii imeni Grekova	Grekov Bulletin of Surgery
Vestnik Leningrad. Univ. Ser. Biol.	Vestnik Leningradskogo Universiteta, Seriya Biologii	Journal of the Leningrad Univ., Biology Series
Vestnik Moskov. Univ., Ser. Biol. i Pochvov.	Vestnik Moskovskogo Universitera, Seriya Biologii i Pochvovedeniya	Bulletin of the Moscow University, Biology and Soil Science Series
Vestnik Oftalmol.	Vestnik Oftalmologii	Bulletin of Ophthalmology
Vestnik Oto-rino-laringol.	Vestnik Oto-rino-laringologiya	Bulletin of Otorhinolaryngology
Vestnik Rentgenol. i Radiol.	Vestnik Rentgenologii i Radiologii	Bulletin of Roentgenology and Radiology
Vestnik Venerol. i Dermatol.	Vestnik Venerologii i Dermatologii	Bulletin of Venereology and Dermatology
Veterinariya	Veterinariya	Veterinary Science
Vinodelie i Vinogradarstvo	Vinodelie i Vinogradarstvo SSSR	Wine-Making and Viticulture
Voprosy Klin.	Voprosy Klinicheskie	Clinical Problems
Voprosy Med. Khim.	Voprosy Meditsinskoi Khimii	Problems of Medical Chemistry
Voprosy Med. Virusol.	Voprosy Meditsinskoi Virusologii	Problems of Medical Virology
Voprosy Neirokhirurg.	Voprosy Neirokhirugii	Problems of Neurosurgery
Voprosy Onkol.	Voprosy Onkologii	Problems of Oncology
Voprosy Pitaniya	Voprosy Pitaniya	Problems of Nutrition
Voprosy Psikhologii	Voprosy Psikhologii	Problems of Psychology
Voprosy Virusologii	Voprosy Virusologii	Problems of Virology
Vrachebnoe Delo	Vrachebnoe Delo	Medical Profession
Zav. Lab.	Zavodskaya Laboratoriya	Factory Laboratory
Zhur. Mikrobiol., Epidemiol. i Immunobiol.	Zhurnal Mikrobiologii, Epidemiologii i Immunobiologii	Journal of Microbiology, Epidemiology, and Immunobiology
Zhur. Nevropatol. i Psikhiat.	Zhurnal Nevropatologii i Psikhiatrii imeni S. S. Korsakov	S. S. Korsakov Journal of Neuropathology and Psychiatry
Zhur. Obshchei Biol.	Zhurnal Obshchei Biologiya	Journal of General Biology
Zhur. Vysshei Nerv. Deyatel.	Zhurnal Vysshei Nervnoi Deyatelnosti imeni I. P. Pavlova	I. P. Pavlov Journal of Higher Nervous Activity
Zool. Zhur.	Zoologicheskii Zhurnal	Journal of Zoology



NOTES of a SOVIET DOCTOR

by

G. S. PONDOEV

Honored Physician of the Georgian SSR

introduction to
the English translation
by

IAGO GALDSTON, M.D., F.A.P.A.

From the preface to the second edition....

This book by Gavriil Sergeevich Pondoev, Honored Physician of the Georgian SSR, published here in its second edition, casts light on the urgent questions of medical conduct in the broad sense of the term. . . . Half a century of service (as a practicing physician) and his work in clinical hospitals, have enabled the author to gather material from extensive observations, and his personal experience has imparted to them a unique value. . . . Young doctors and medical students will discover in G. S. Pondoev's book the answers to many troubling questions of vital importance—questions that will come up not only at the beginning of independent practice, but also later on. . . .

Professor D. G. Mamamtavrishvili,
Honored Scientist

CONTENTS

- The Vocation of the Doctor
- Not So Very Long Ago
- Medicine—Art or Science?
- Books, Lectures, Examinations
- The Doctor and the Patient
- At the Patient's Bedside
- The Doctor's Mode of Thought
- Anamnesis, Diagnosis, Prognosis
- Prophylaxis and Treatment
- Specialists
- Doctors and Medicine in the Field
- The Doctor's Mistakes
- Medical Ethics and Medical Secrecy
- The Doctor and the Group
- Homeopathy
- The Quack

"...A work of many dimensions..." this exposition of Russian medical conduct and ethics will prove to be of absorbing interest and great value to both the medical and lay public.

Dr. Galdston

6"x9", 246 pages **$4.95**

c/b CONSULTANTS BUREAU
227 W. 17 ST., NEW YORK 11, N. Y.

INTRAARTERIAL INFUSION OF PROCAINE

IN THERAPEUTIC PRACTICE

by N. K. Gorbadei

Translated from Russian

This English translation of Dr. Gorbadei's book includes as an appendix a report by I. I. Velikanov on treatment of hypertensive patients with intraarterial procaine infusions. Dr. Velikanov's report was first published in the Bulletin of the Academy of Sciences of the Kazak SSR.

Cases of peptic ulcer dating back to the siege of Leningrad responded to a series of infusions of procaine into the arteries, so that the patients were able to resume their normal activities, according to the author, Dr. N. K. Gorbadei who has reported on 177 cases of peptic ulcer treated at the Leningrad Sanitary-Hygiene Medical Institute.

CONTENTS

Introduction

Intravascular Infusion of Procaine in Therapeutic Practice (a survey of the literature) (A Short Account of the Properties of Procaine; Intravenous Procaine Infusions; Intraarterial Procaine Infusions.)

The Mechanism of Action of Procaine

The Technique of Intraarterial Infusion of Procaine

The Treatment of Peptic Ulcerations by Intraarterial Procaine Infusions [Clinical Features of the Patients Under Investigation; Influence on Subjective Symptoms; Roentgenological Findings During Treatment; Acid-secreting Function of the Stomach During Treatment; Changes in the Blood Picture; Changes in the Zones of Cutaneous Sensation; State of the Cardiovascular System (Electrocardiography and Plethysmography) During Treatment.]

The Treatment of Patients Suffering from Bronchial Asthma by Intraarterial Procaine and Penicillin

The Treatment of Coronary Insufficiency by Intraarterial Procaine Infusions

Intraarterial Procaine Infusions in the Treatment of Infectious Polyarthritis

Indications for and Contraindications to the Use of Intraarterial Procaine Infusions in the Clinical Treatment of Internal Diseases

Conclusion

Bibliography

Soviet Literature
Non-Soviet Literature

Supplement

The Treatment of Patients with Hypertension by Intraarterial Infusion of Procaine Solution. I. I. Velikanov

135 pages **$7.50**

CONSULTANTS BUREAU
227 W. 17 ST., NEW YORK 11, N. Y.